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Doctoral Course in
“ Environmental and Energy Engineering Sciences ”

XXXI cycle

Dissertation title
"Energy and material recovery from high-loaded organic substrates: A *territory-oriented* approach"

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Year 2019

To all the people who helped and supported me throughout this long Ph.D. trip, starting from my family, and going on with CAFC S.p.A. and Udine University colleagues

Contents

Abstract

This Ph.D. research was aimed at liquid organic substrates valorisation, by means of energy and material recovery. The mountain area of Friuli-Venezia Giulia region was selected as case-study: Cheese Whey (CW), coming from local dairies, condensate Pulp and Paper (P&P) wastewater, Organic Fraction of Municipal Solid Waste (OFMSW), brewery organic waste (spent grain, yeast, whirlpool residue, end-of-fermentation beer) and slaughterhouse liquid waste were selected for Anaerobic Digestion (AD) process application (with energy recovery, through biogas), as well as for resource recovery (through valuable compounds extraction from CW and fertilizers production from OFMSW).

The work started with a preliminary literature study, followed by physico-chemical characterization of the substrates (using traditional and macromolecular parameters), BMP tests (useful to estimate methane yields of each substrate, in different operating conditions), continuous UASB tests (performed on a pilot-UASB unit, located in Tolmezzo WWTP), and it was then completed with an energetic analysis, as well as with some final remarks and suggestions, to improve actual management strategies of this wastes.

The Ph.D. dissertation starts with a general introduction, aimed at describing EU perspective in renewable energy (focusing in particular on biogas and biomethane) and waste management, to contextualize the study. Given the increasing importance of biomethane, the currently applied technologies for biogas upgrading are briefly discussed, as well. Then, UASB anaerobic treatment, as an interesting process for energy recovery from high-loaded industrial wastewater, and Tolmezzo WWTP (143,000 PE) are described, as the starting point of the work.

Successively, the analysed substrates are introduced (preceded by a general literature study), and the obtained characterization results are presented, also in comparison with literature evidences. The results from Biochemical Methane Potential (BMP) tests follow: these data were useful to estimate potential

methane yields and maximum methane fluxes, as well as to introduce continuous UASB tests, that were conducted on a pilot-unit, located in Tolmezzo WWTP.

In the final chapter, some energetic and material recovery considerations are drawn, in light of the results of the study, considering, where available, the actual energetic costs in selected real plants and suggesting, for each substrate, an optimization route, both from an economical and an environmental perspective.

The results underlined that a high potential is present for biogas production, in particular for dairies: CW can be successfully digested, and, if performed at plant level, AD process can provide most of the electricity and heat needed by the process. Moreover, the installation of simple digesters can allow to reduce pay-back time of the investment cost, allowing also for small plants to sustain the initial expense. In larger dairies, instead, resource recovery should be privileged, due to the extra income that could be provided by the obtained products. Furthermore, ultrasound (US) pre-treatment was shown to be effective in increasing biogas yields, but only at low applied US energy.

OFMSW can be separated into a liquid fraction, highly biodegradable and having good methane potential, and a solid fraction, that is easily stabilized through composting units. Given the general low amount of available organic waste in the analysed territory, co-digestion of OFMSW liquid fraction with other substrates, such as excess sewage sludge, can be an interesting option, to increase biogas yields and obtain a co-digestion mixture having optimum characteristics for AD process.

Condensate water is a highly concentrated P&P wastewater, and is amenable to be pre-treated using UASB technology; this can reduce organic load to the aerobic basins of Tolmezzo WWTP, leading to a significant energy saving for aeration.

Brewery organic waste is characterized by a pool of different substrates, including spent grain (trub), yeast, whirlpool residue and end-of-fermentation beer. A good potential for biogas production was shown to be present mainly in spent grain and yeast, while lower yields were obtained from whirlpool and beer. The addition of little amounts of biochar and granular activated carbon increased obtainable methane yields from whirlpool and yeast in a significant way (more than 35%), so a synergistic effect between biomass plants and processing plants can be achieved, improving energy production. Co-digestion of brewery organic substrates at plant level can be successfully performed, and positive mutual effects between yeast and AD biomass should be evaluated, that could potentially further enhance methane yields. Given the high energy demand of this plants, in particular for thermal energy, AD process appears to be a good solution to reduce operating costs of local breweries.

Slaughterhouse waste, finally, is a harsh substrate, difficult to hydrolyse in AD processes, and rich of proteins and fats: in order to be successfully treated using AD process, efficient pre-treatments should be investigated. Moreover, sanitary protocols have to be followed, for its proper management. Again, it could be interesting to evaluate the effects of co-digestion with complementary substrates, rich in C, that could allow easy operations and increase in methane yields.

Chapter 1

Introduction

In this chapter, the general framework in which this research was conducted will be described; in particular, the EU perspective in waste management will be discussed, with particular focus on anaerobic digestion (AD) technology. As waste-water (WW) treatment requires huge energy inputs, some general considerations about energy consumption in waste-water treatment plants (WWTPs) will be made; in addition, the currently available technologies to upgrade biogas to biomethane will be briefly introduced, given the great expansion of biomethane market, that is being observed nowadays in EU. Moreover, some statistical data from European Biogas Association (EBA) about AD diffusion in EU will be presented. Finally, the aim of the current Ph.D. research, that was focused on energy and material recovery from high-loaded liquid substrates, present in the mountain area of Friuli-Venezia Giulia region, will be stated.

1.1 EU perspective in waste management

In recent years, due to urgent environmental problems, such as climate change, temperature increase and growing pollution levels, a great effort has been done by political and economic authorities to promote a shift towards a more sustainable management of natural resources. In particular, the concept of circular economy raised attention in EU proposals, leading to the development of well-structured funding programs, such as Horizon 2020 [1].

Actually, EU encourages the transition to a new economic paradigm, where the value of products, materials and resources is maintained in the system as

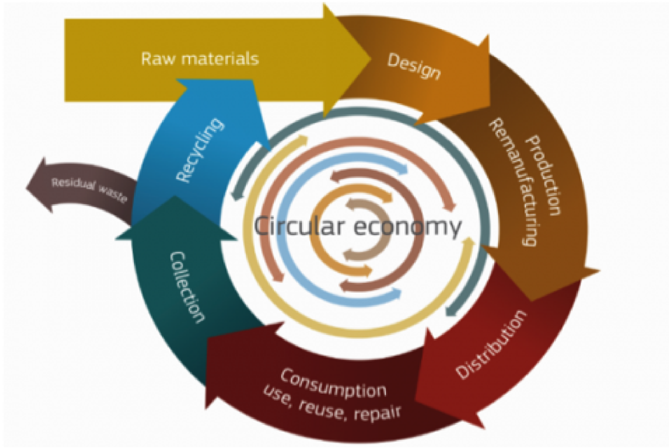

Figure 1.1: EU circular economy perspective [2]

long as possible, while waste generation is minimised [1]. The circular economy (fig. 1.1) is supported in each step of the value chain: from production to consumption, repair-remanufacturing, waste management, and secondary raw materials production, that are finally fed back into the system, closing the cycle [1].

Waste management plays a central role in circular economy; a cascade hierarchy (fig. 1.2) is established, starting from prevention and preparation for reuse. Recycling and energy recovery follow next, while, obviously, landfill disposal is strongly discouraged [3].

Furthermore, the market for secondary raw materials and water reuse is boosted. In this category, particular importance is given to recycled nutrients: in fact, their sustainable use in agriculture reduces the need for mineral-based fertilisers, whose production has negative environmental impacts, basically depending on imports of phosphate rock, that is a very limited resource at global level [1] (as shown also in recent literature studies, like [4]).

In particular, as for biodegradable organic waste, that is also called OFMSW (Organic Fraction of Municipal Solid Waste), when coming from household facilities, an increased production of waste-derived biogas is encouraged, for the purposes of cogeneration, injection into the gas grid (as biomethane), use as transport fuel and fertilisers production, mainly through AD technology [3].

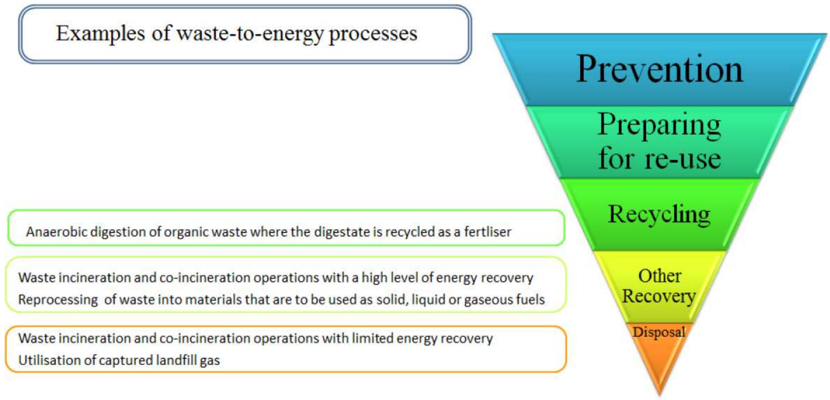

Figure 1.2: EU waste hierarchy in the circular economy approach [3]

Finally, in addition to water-efficiency measures, another noticeable aspect, that is specifically mentioned in EU acts, is treated wastewater reuse, that must be safe and cost-effective, and that should alleviate, in the future, the pressure on over-exploited water resources [1].

The importance of anaerobic treatment of solid waste and WW showed its great potential in last decades, to reduce GHG emissions, and to properly valorise organic waste from agriculture and industry. Historically, waste treatment in EU has undoubtedly evolved, starting from simple composting process of organic waste, localized at farm level, to the development of centralized AD plants (working not only with single substrates, but also in co-digestion) and, more recently, to biogas up-grading technologies, whose high-value final product is bio-methane, that can fully replace fossil-based natural gas.

Hopefully, as sustained by EU, the next step will be bio-refineries development, able to produce a large spectrum of commercial products, currently manufactured using fossil fuels (mainly oil-derived, such as plastics) [1]. The concept of bio-refinery is exemplified in fig. 1.3, where the products that can be obtained in an ideal bio-based plant are listed, starting from alternative fuels (for example, ethanol and biodiesel), to bulk chemicals, plastics and fine chemicals.

Bio-refining was defined from the International Energy Agency as “the sustainable processing of biomass into a spectrum of marketable products and energy” [6]. The raw materials have an organic biological origin and can be

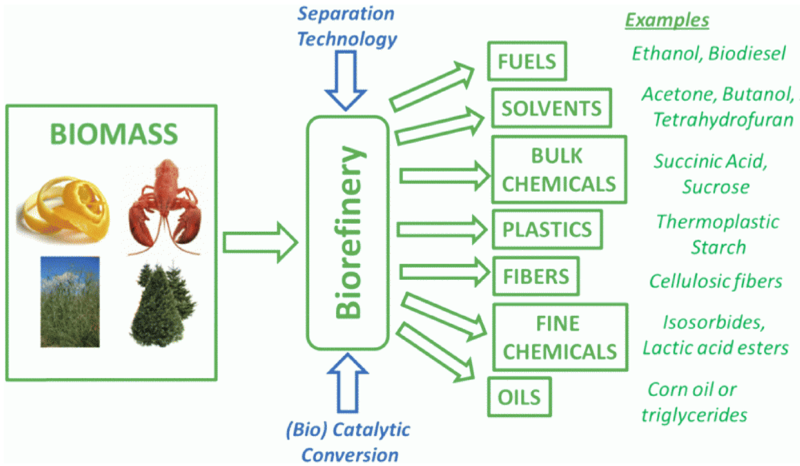

Figure 1.3: Biorefinery concept [5]

grown and harvested many times within a short span of time, and any eventual conflict with food production is excluded.

The concept of bio-refinery is similar to a petrochemical refinery, where biomass plays the same role as oil. However, despite many of the separation processes and unit operations are the same as traditional petrochemical plants, much still has to be done in terms of process development and design [7]. In addition, several issues need to be solved for bio-refineries full-scale application, such as the combination of supply and distribution chains with the production process [8].

1.2 Energy consumption in WWTPs

The general considerations about EU perspectives on waste management need to be actualized in strongly heterogeneous fields, from industrial plants, that are responsible for proper waste and WW management, to Integrated Water Service (IWS) authorities, that have the fundamental role of preserving water quality, as well as reducing environmental risks, that come from poorly treated WWTP effluents, by means of an efficient and energy-sustainable WW treatment.

WWTPs were generally designed to meet certain effluent requirements, without any major energetic consideration [9]; so, WWTPs were hardly ever designed with energy efficiency in mind [10]. This attitude was however changing in recent years, to reach the 20-20-20 goals, defined for Climate and Energy by Directive 2009/28/E [11]. Moreover, considering both the improvement of pollutants removal and the diffuse centralization trend of WWTPs in highly populated metropolitan areas, the optimization of WWTPs energy efficiency is nowadays a crucial issue for managing companies [12].

In a conventional WWTP, about 25-40% of global operating costs can be ascribable to electric energy consumption; this value is reported in literature to be in the range of 0.3-2.1 kWh/m^3 of treated wastewater [13]. The main contributes to energy consumption in a conventional WWTP are typically mixed liquor aeration (55-70%), primary and secondary settling with sludge pumping (15.6%) and sludge dewatering (7%) [10]. In an advanced biological treatment, with nutrients removal and filtration, an increase up to 50% in electricity consumption can be observed, in comparison with a conventional activated sludge process [10]. Other important issues, that can decrease energy efficiency, are the incorrect maintenance of electro-mechanic devices, the presence of infiltration surface rainwater in the sewer network, and any anomalous hydrodynamic behaviour of the reactors [12].

In literature, energy efficiency optimization in WWTPs is a rather popular topic; as an example, a remarkable work, reported in [12], presents a multi-step methodology to evaluate the energetic consumption of a large Italian WWTP (2.7M PE). Each phase of the process scheme was considered, to get specific electricity consumption for all electro-mechanic devices. Successively, the total electric energy demand of the plant was evaluated (66.8 GWh/y, about 50% from aeration tanks) and four specific energy consumption indexes were introduced, relating the electric energy demand to treated PE, WW volume, removed COD and TN, in order to compare the selected WWTP with other plants. Furthermore, the thermal energy demand of the plant was estimated (49.2 GWh/y, more than 93% from sludge line), and, finally, some energy optimization solutions were suggested, to decrease operating expenses.

This is a meaningful example of the work that IWS authorities need to face, to increase WWTPs energy efficiency, starting from largest plants, which are more easily controlled: WWTP management, in the future, will need to shift from an approach solely oriented to effluent limits respect, to a broader view, that should consider the energetic optimization of each process phase. Moreover, resource recovery from WW and sludge will allow to reduce the environmental impact of the plants; this new perspective is well summarized in the acronym WRRFs (Water Resource Recovery Facilities) [14], that is progressively replacing

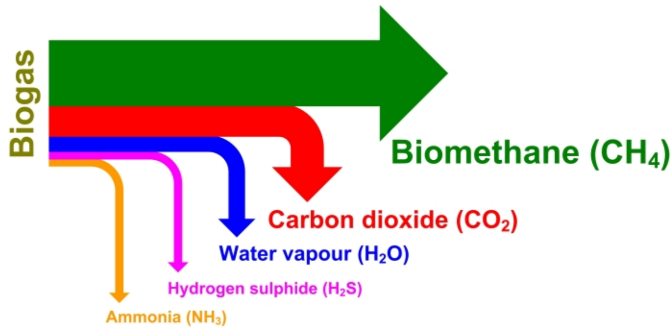

Figure 1.4: Concept of biogas upgrading [17]

the "traditional" acronym WWTPs, to underline the paradigm shift from basic WW treatment to a more complex platform, where energy and resources are fully recovered, reducing GHG emissions and effluents toxicity, leading to innovative platforms, that preserve environmental quality with a neutral (or even positive) energy balance.

Finally, another aspect that is worthwhile to mention is the increasing diffusion of WWTP mathematical models, that can help to achieve a deeper knowledge of the processes and can be very useful in identifying the tricky issues: a proper model application in existing WWTPs generally allows to reduce energy consumption, achieving, at the same time, a higher general process efficiency.

1.3 Biogas upgrading technologies

As was briefly highlighted in the previous paragraphs, biogas is playing a key role in the emerging renewable energy market [15]. In fact, it is estimated that a major part of the EU-27 renewable energy target by 2020 will be met by bioenergy, at least 25% of which will be biogas [16]. In addition, the global capacity for power generation from commercial biogas facilities will more than double over the next decade, from 14.5 GW in 2012 to 29.5 GW in 2022 [15]. In large WWTPs, biogas is already produced from AD of excess sewage sludge and, in some cases, co-digestion with other organic substrates is performed, to increase energy production.

It is well known (fig. 1.4) that biogas is mainly composed of CH_4 and CO_2; small amounts of water vapour, NH_3, H_2S, H_2, O_2, N_2, CO and siloxanes are

Table 1.1: Comparison between biogas and natural gas characteristics [18]

Parameter	Biogas from AD	Natural gas
LHV (MJ/Nm^3)	23	39
CH_4 (% mol)	60-70	85-92
Heavy hydrocarbons (% mol)	0	9
H_2 (% mol)	0	-
CO_2 (% mol)	30-40	0.2-1.5
H_2O (% mol)	1-5	-
N_2 (% mol)	0.2	0.3
O_2 (% mol)	0	-
H_2S (ppm)	0-4,000	1.1-5.9
NH_3 (ppm)	100	-
Total Cl (mg/Nm^3)	100	-

present, as well. Some of the impurities may have significant negative impacts on the utilisation system, such as corrosion, increased pollutants emissions and hazards for human health [18]. Moreover, it must be considered that raw biogas has a consistently lower calorific value, if compared to natural gas. These observations are further detailed in tab. 1.1, where the chemical characteristics of natural gas and raw biogas are compared.

In order to increase the calorific value and reduce unwanted components in biogas (such as H_2S), it is indeed important to clean raw gas and upgrade it to a higher fuel standard. This process is called biogas cleaning and upgrading [19]. Upgrading biogas to biomethane is one of the technologies that actually attract greater interest in the bioenergy industry. In Europe, the total installed capacity for biogas upgrading grew from less than 10,000 Nm^3/h (raw gas) in 2001 to over 160,000 Nm^3/h (raw gas) in 2011 [20]. A large number of technologies for biogas cleaning and upgrading have been developed up to date, and some of them are commercially available.

Actually, the main technologies, currently applied in this field, are:

- Water scrubbing (eventually with regeneration);
- Cryogenic separation;
- Physical absorption;
- Chemical absorption;

- Pressure Swing Adsorption (PSA);
- Membrane separation [18].

An important factor, that must be carefully considered when choosing between these technologies, is the loss of methane in the process, together with its contribution to green-house gases (GHG) emissions [18].

It has been well recognised that the selection of the "right" upgrading technology must be site-specific and case-sensitive, depending on local circumstances and specific requirements by end-use purposes, as well as related regulations [18]. The main technologies, mentioned above, will be briefly described in the following, considering both technical and economic aspects.

1.3.1 Water scrubbing

Water scrubbing, whose general scheme is reported in fig. 1.5, exploits water as scrubbing agent, to remove impurities from biogas: CH_4 solubility in H_2O is much lower than that of CO_2. H_2S, being more soluble than CO_2, can be as well eliminated, even if H_2S pre-separation is normally necessary, because it is poisonous and creates corrosion problems. Water scrubbing can give a CH_4 purity of 80-99%, depending on the volume of non-condensable gases, such as N_2 and O_2, that cannot be separated from CH_4 [18]. It is possible, as well, to achieve a high purity (up to 80-90%) side-stream of CO_2; CH_4 losses are usually in the range of 3-5%, even if suppliers claim that they can be controlled below 2% [21]. Energy consumption is mainly related to raw gas compressing and water processing by circulation pumps. If also air stripping is included, it should be reminded that the air fan for water regeneration also consumes some electricity.

1.3.2 Cryogenic separation

Cryogenic separation (fig. 1.6) takes advantage of the different condensing temperatures of the gases, to separate CO_2 from CH_4; to avoid freezing, water and H_2S need to be pre-separated out. N_2 and O_2 can also be removed, differently from water scrubbing. A large amount of energy (5-10% of produced biomethane) is required in the process, because raw gas must be compressed to high pressure (up to 200 bar) to be cleaned [23]. The main advantages of cryogenic separation are the production of liquid and high-purity biomethane, and the limited losses of CH_4 (lower than 1%). Also, high-purity CO_2, up to 98%, can be produced [24].

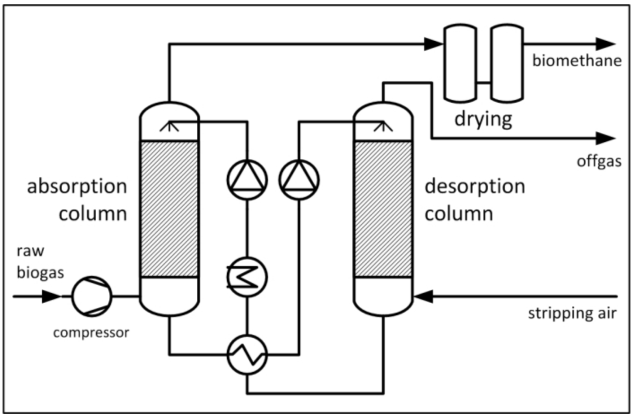

Figure 1.5: Water scrubbing (biogas upgrading) [22]

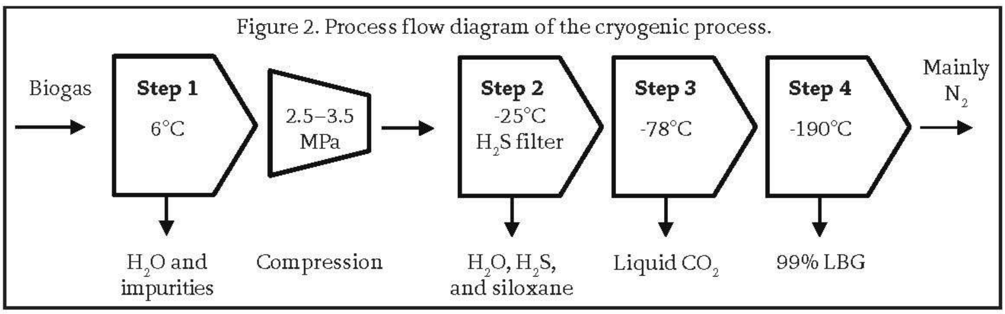

Figure 1.6: Cryogenic separation (biogas upgrading) [25]

1.3.3 Physical absorption

Physical absorption is based on the same principle as water scrubbing, but, instead of water, organic solvents (such as methanol) are used to absorb CO_2. The main technical characteristics are indeed similar to water scrubbing (inability to remove N_2 and O_2, high CH_4 losses). Since CO_2 has a higher solubility in organic solvents, the upgrading system can be more compact, producing a high-purity CO_2 stream, and part of the pumping work can be avoided. H_2S pre-separation is again required, because it is difficult to regenerate H_2S from the solvent. The energy consumption of physical absorption is comparable to that of water scrubbing; in addition to electricity, heat, at T of 55-80 °C, is needed, to regenerate the solvent [18].

1.3.4 Chemical absorption

In this case, differently from physical absorption, chemical reactions between absorbed substances and solvent occur; the use of chemical solvents is generally preferred over physical methods when CO_2 concentration is low. The scheme of a chemical absorption process is reported in fig. 1.7; amines are widely employed as reagents, as they selectively react with CO_2, with no CH_4 losses. However, simulations show that more than 4% of CH_4 can be lost, due to the dissolution in water [27]; this loss further affects the purity of CO_2 stream, which contains about 93% CO_2 and 6% CH_4 [24]. Another downside of this technology is related to energy consumption: a large amount of high-temperature heat is needed to regenerate chemical solvents.

1.3.5 Pressure Swing Adsorption (PSA)

PSA process (fig. 1.8) is based on selective adsorption of gas molecules on solid surfaces, according to the molecular size. In fact, CH_4 can be separated from N_2, O_2, CO_2, since CH_4 molecule is larger than the other gas molecules [28]. H_2S adsorption is irreversible, and so H_2S is considered toxic to PSA, and needs to be pre-removed [24]. CH_4 concentration after upgrading is typically 96-98%, and methane losses are about 2-4% [23]. More CH_4 is lost at higher purity requirements and, due to the high CH_4 concentration, the vent gas has to be properly treated, before being released to the atmosphere [29].

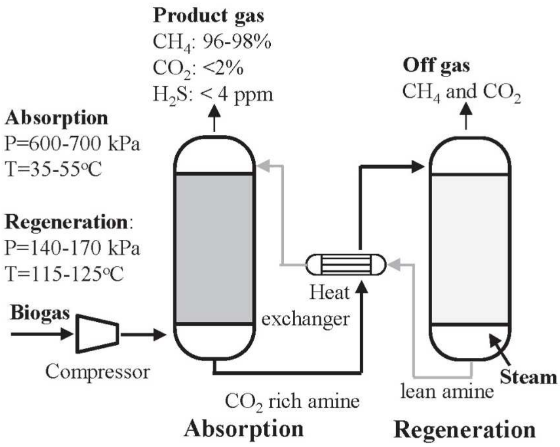

Figure 1.7: Amine absorption (biogas upgrading) [26]

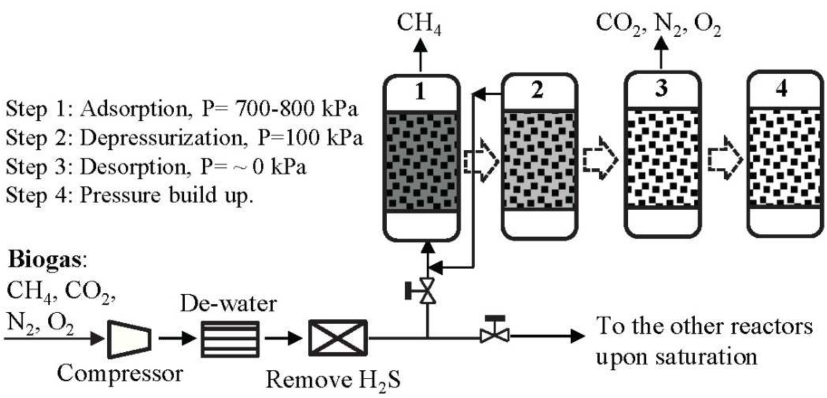

Figure 1.8: Pressure Swing Adsorption (biogas upgrading) [26]

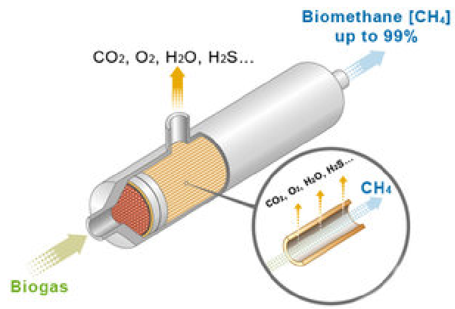

Figure 1.9: Membrane technology (biogas upgrading) [30]

1.3.6 Membrane technology

Membrane technology is a separation method at molecular scale, and has a number of merits, including low cost, energy efficiency and ease of process. CO_2 and H_2S, as well as O_2 and H_2O, pass through the membrane to the permeate side (fig. 1.9), while CH_4 is retained on the inlet side. Since some CH_4 molecules can pass through the membrane, if a high CH_4 purity is needed, large CH_4 losses are encountered. The most suitable commercial membranes are polyimide and cellulose acetate-based membranes. Process optimization can lead to a CH_4 purity of 98%, with 99% CH_4 recovery, while electrical energy consumption is around 0.3 kWh/m^3 [18].

1.4 AD plants actual situation in Europe

After having introduced relevant aspects related to EU perspectives in renewable energy, and in particular biogas, in this paragraph some statistical data about AD technology application throughout Europe will be briefly presented. EBA (European Biogas Association) publishes yearly a statistical report of the current situation in AD plants in Europe; some of the most recent data are described hereunder, to give a general overview.

As can be seen from fig. 1.10, the leading country in Europe, as for AD technology application, is Germany, where more than 10,000 AD plants are actually operating. Italy comes second, with more than 1,500 AD facilities, followed by France and Switzerland. So, it can be inferred that there is quite some unexploited potential for further AD improvement, because of the abundant

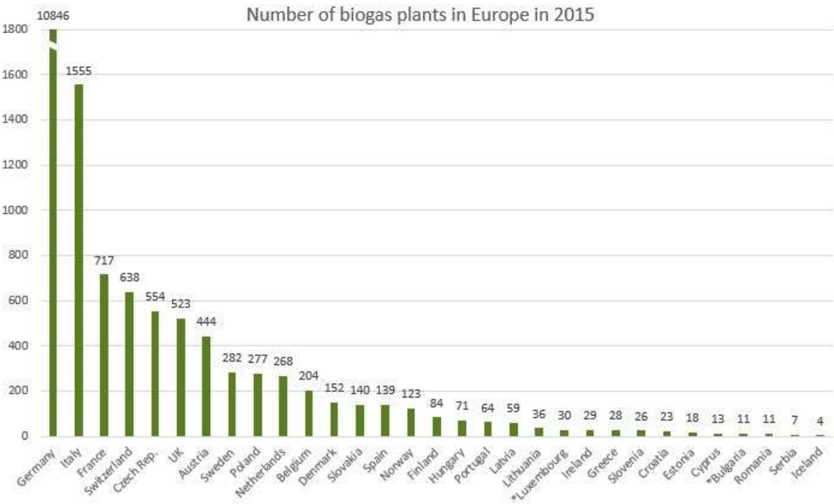

Figure 1.10: AD plants distribution in EU in 2015 [31]

presence (in particular in the Mediterranean area) of organic substrates, amenable to be anaerobically valorised. Moreover, EU incentives boost for an additional development of a robust and mature technology, as AD proved to be, reducing the dependence on extra-EU fossil fuels, that are going to be more and more costly in the future.

Biomethane, more than simple biogas, represents the technology with the largest growth potential, because of its capability to penetrate monopolistic fossil market in transports, helping to increase renewable fraction in the sector. Fig. 1.11 represents the increase in biomethane facilities in the years 2011-2015: a mean 25-30% increase was observed from one year to the successive one, and a greater increase is expected in next years, following EU policies towards an increase in sustainable and renewable energy sources, immediately applicable to full-scale (that is, having a Technology Readiness Level, TRL, of 9). It can be highlighted, in the end, that EBA conference, that is organized every year to spread technical knowledge and facilitate collaboration between political authorities and companies, was mainly focused, in 2016 and 2018, on biogas upgrading, being "Greening the gas" the main theme of the event.

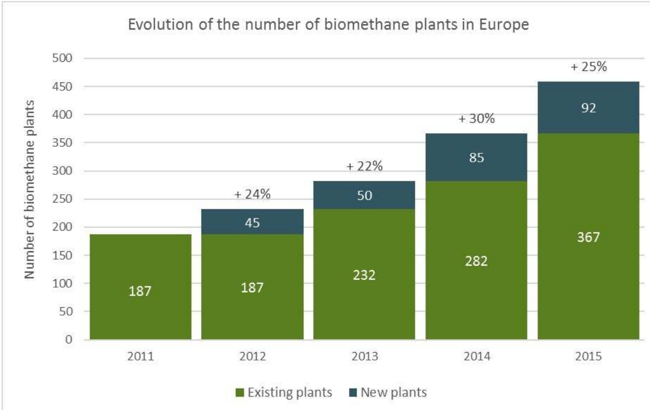

Figure 1.11: Growth in biomethane plants in Europe from 2011 to 2015 [31]

1.5 Aim of the work

In this perspective, the work that was carried out in this Ph.D. was focused on energetic valorisation (through AD) and material recovery from high-loaded liquid substrates, in order to propose a solution for selected waste and WW treatment, able to improve energy, environmental and economic balance, both for IWS authorities and local industrial facilities.

In particular, Tolmezzo WWTP (143,000 PE), located in the mountain area of Friuli-Venezia Giulia region, was selected as a case-study. This plant is provided of a high-velocity anaerobic up-flow anaerobic sludge blanket (UASB) reactor; the technical feasibility of anaerobically digesting selected organic substrates, available in the analysed territory, was investigated, taking into account the specificity of each stream, and evaluating the impact of biogas on energetic need from the plants. The selected substrates where chosen among the matrices whose management and disposal costs actually represent an issue for the producing plants.

In particular, the following matrices were selected, basing on a preliminary literature work:

- Cheese Whey (CW), coming from local cheese factories (first whey was collected and analysed separately from second whey);
- Condensate P&P wastewater, coming from lignin-sulphonate concentration

process;

- OFMSW leachate, originating from percolation of source-sorted OFMSW;
- Brewery organic waste (spent grain, whirlpool residue, yeast, beer);
- Slaughterhouse liquid waste, generated in process activities.

The work proceeded through the following phases:

1. Literature study of selected liquid organic substrates;
2. Contact with local facilities, followed by samples withdrawal and physicochemical characterization (using traditional and macromolecular parameters), to underline the main substrate properties that influence AD process;
3. Laboratory batch anaerobic digestion tests, performed using Automatic Methane Production Test System (AMPTS, Bioprocess) equipment, to establish CH_4 production potential from each substrate, using standardized protocols;
4. Continuous UASB tests, executed, as for CW, OFMSW leachate and condensate water, using a pilot-UASB reactor, to verify the actual feasibility of a UASB treatment, at proper operating conditions;
5. Energetic and material recovery considerations, in particular for CW, OFMSW and brewery waste, with analysis of obtainable methane yields at full-scale AD, and final conclusions, in light of the obtained results.

Chapter 2 will present the case study (Tolmezzo WWTP), focusing in particular on UASB section, and some general considerations about anaerobic UASB treatment will be made. A particular focus will be put on UASB (and in general AD) process modelling, given the actual possibility of mathematically describing these processes.

Chapter 3 will highlight the peculiar characteristics of each selected matrix, starting from literature evidences, and will present the results of physicochemical characterization, comprising both traditional, macromolecular and elemental parameters.

Chapter 4 will be focused on BMP tests, presenting the used laboratory equipment and the obtained results, that were used to plan the successive phases. Ultrasound (US) pre-treatment was tested on CW, in order to evaluate a possible increase in biogas yield, through disaggregation of large CW molecules. Biochar and granular activated carbon, instead, were added to selected brewery waste (spent yeast and whirlpool) to enhance methane yields. In addition, a kinetic analysis was performed on the obtained data, to get some meaningful parameters on substrates hydrolysis.

Chapter 5 will present UASB-pilot plant, that was built in Tolmezzo WWTP, and will describe the results obtained on the different substrates (diluted CW, OFMSW leachate, condensate water). The results from odour campaign and respirometric COD fractioning (executed on condensate water) will be presented, as well.

Chapter 6 will conclude the work with some energetic and material recovery considerations, in particular on CW, OFMSW and brewery waste, suggesting a new treatment strategy, that should privilege resource recovery, as well as energy valorisation, rather than simple utilisation as a feed in animal farms, as it is done nowadays, and some suggestions for further studies on this topic will be stated.

Chapter 2

UASB treatment: The case-study

2.1 Geographic overview

Tolmezzo (Ud) is a little municipality of about 10,000 inhabitants, located in the mountain area of Udine province (fig. 2.1); in this area, Integrated Water Service (IWS), that includes, as known, freshwater supply, sewers and WWTPs management, is actually held by CAFC S.p.A., that is the reference water utility for most of the province (121 out of 135 villages), excluding Cividale area. CAFC is also the most important IWS authority in Friuli-Venezia Giulia Region. The total managed area by CAFC is 4,594 Km^2; in this vast territory, an analysis of the actual WW treatment situation (fig. 2.2) shows that a huge amount of WWTPs is present (more than 500), mainly because of the scattered demographic distribution: a high number of little villages is present, each one with his own WWTP.

This is merely an inheritance of the first sanitation techniques, applied after "Merli" Law of 1976, that introduced some basic principles to prevent the release of untreated WW in the environment; the approval of this law, in Friuli-Venezia Giulia Region, actually coincided with the terrible earthquakes of May and September 1976, that caused the loss of hundreds of human lives, as well as huge damages to local infrastructures and buildings. The majority of the existing WWTPs were built during the reconstruction, that followed in the successive years, and, in recent decades, they have been started to be

Figure 2.1: Localization of Tolmezzo (Ud) in Friuli-Venezia Giulia region [34]

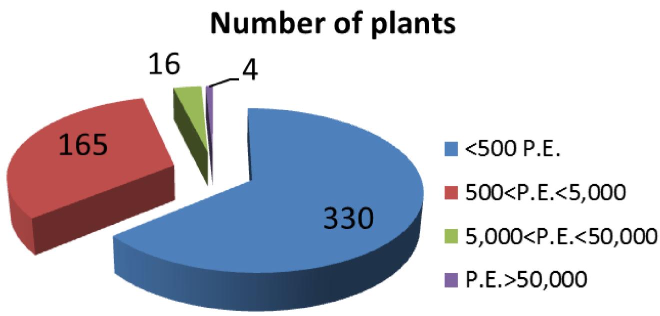

Figure 2.2: WWTPs in CAFC S.p.A. territory, divided by potentiality class (expressed as P.E.)

progressively revamped, given the actual concern not only for C removal, but also for nutrient (N and P) uncontrolled release in the environment.

Nonetheless, actually most of the existing plants in the province (fig. 2.2) have a very limited potentiality (<500 PE), merely consisting of simple Imhoff tanks; a limited number of medium potentiality plants (5,000<PE<50,000) is present, while only 4 big plants exist: S. Giorgio di Nogaro (700,000 PE), Udine (200,000 PE), Tolmezzo (143,000 PE) and Lignano (86,400 PE) WWTPs. It should be pointed out that a complete shut off of little plants is not feasible, especially in the mountain area, because many kilometers of sewer pipes should be laid, to collect and transport all WW to largest plants, given the long distance from a village to the nearest one.

S. Giorgio plant has the highest potentiality in Udine province, because it was designed to treat WW coming from an important industrial area (Aussa-Corno), even if, after economic crisis in 2008, a significant number of industries reduced their activity, so the plant is currently treating only about 200,000 PE.

Lignano plant is characterized by an extreme seasonal variability, due to the tourist fluxes, that are very consistent in Summer: as an example, in August the plant needs to face an organic load that is even 10 times higher than in the Winter period.

Udine plant, instead, is a typical municipal plant, characterized by a substantial absence of industrial WW and mixed sewage network.

2.2 Tolmezzo WWTP

2.2.1 Process scheme

Tolmezzo WWTP (fig. 2.3) is a predominantly industrial plant, that treats 4 distinct WW streams, 3 of them coming from the neighbouring Mosaico P&P factory (namely condensate water, whitening water and process water); municipal WW, that is the fourth stream, is coming from Tolmezzo, Amaro and Villa Santina sewers. Globally, plant potentiality is 143,000 PE, where 128,000 PE come from the P&P factory, while the remaining 15,000 PE are ascribable to civil wastewater [35]. The treatment process, summarized in fig. 2.4, mainly consists of the following steps [35]:

- Urban wastewater pre-treatment (screening, sand and oil removal);
- UASB anaerobic pre-treatment of condensate water;

Figure 2.3: Tolmezzo (Ud) WWTP

- Aerobic activated sludge treatment of all the WW streams (pre-treated condensate water, whitening and process P&P WW, urban WW) followed by secondary clarification;
- Tertiary physicochemical treatment of coagulation-flocculation;
- Sludge aerobic digestion, thickening and mechanical de-watering.

Some meaningful pictures from the plant could be reported: in fig. 2.5, air diffusers arrangement in biological tanks is showed, while in fig. 2.6 sludge extraction system from secondary clarifiers is visible.

The peculiarity of this plant is obviously the prevalence of P&P wastewater, that is characterized by low nutrients concentration: as a consequence, a specific N and P removal is not needed, so aerobic basins have just the function of organic C abatement (total oxidation). Classical municipal WWTPs, instead, nowadays need to perform nutrient, as well as carbon, removal: this is typically done by alternating anoxic, anaerobic and aerobic phases, where NH_3 is firstly oxidized to NO_3^- (through intermediate production of NO_2^-), in the nitrification process, and then NO_3^- is reduced to N_2, through denitrification. Also, biological or chemical P removal is usually performed.

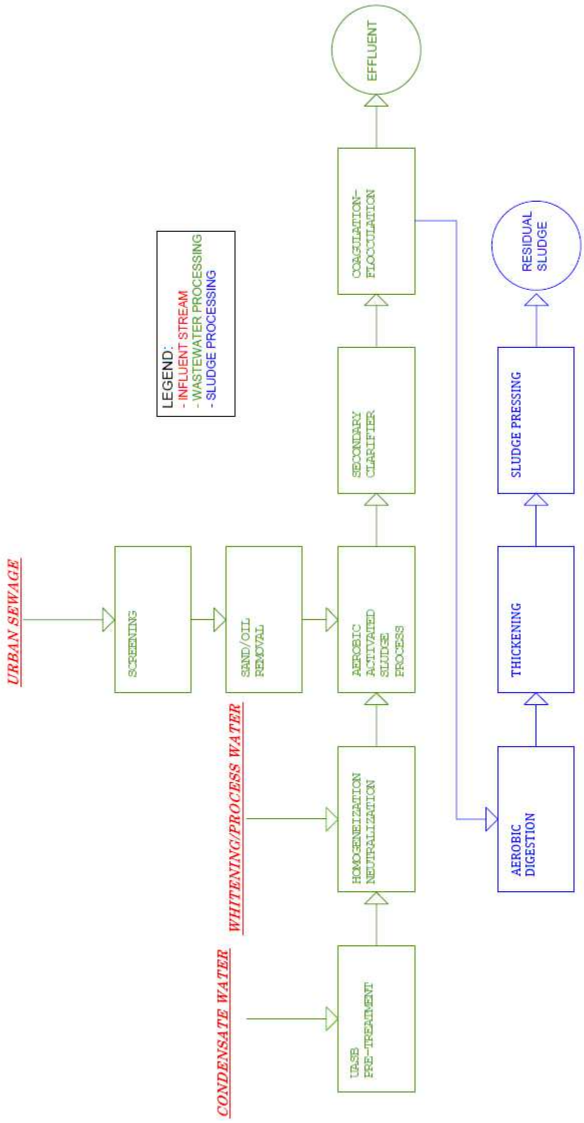

Figure 2.4: Tolmezzo (Ud) WWTP process scheme

Figure 2.5: Air diffusers arrangement in Tolmezzo (Ud) WWTP

Figure 2.6: Sludge extraction system in Tolmezzo (Ud) WWTP clarifiers

2.2.2 Influent streams characteristics

Condensate water comes from lignin-sulphonate concentration, performed in Mosaico P&P factory, that is a process aimed at increasing solution solid content (from 10%, to values as high as 55%), allowing to make it marketable. In fact, lignin-sulphonate is a binder and can be used, as an example, for animal feed pellet production, or other zootechnical applications [33]. Condensate water is characterized by the highest COD concentration of all the Mosaico P&P streams (COD up to 3.5-4.0 g/L), negligible solid matter, acidic pH, scarce nutrient (N and P) concentration, sulphur compounds presence, high T (30-35 °C). However, a detailed characterization of this stream, for the purposes of this work, will be presented in Chapter 3.

Whitening water comes from pulp bleaching, where several chemical agents (such as hydrogen peroxide, sodium hypochlorite, sodium hydroxide and sodium silicate) are used in different stages to whiten the paper product. This stream is characterized by lignin presence (highly refractory to biodegradation, that means low BOD/COD value), as well as high organic and hydraulic loads [33].

Process water, finally, originates from paper production, where internal and external pulp are mixed and inert substances, starches and adhesives are added, to improve mechanical and chemical characteristics of the product. Paper production process is then completed in the paper machine, where water is extracted from cellulose fibers, by means of hydrodynamic, mechanical and thermal steps. Process water is characterized by the majority of hydraulic flowrates, but lower COD and pollutants concentration, if compared to the other P&P streams [33].

Urban WW, finally, shows the typical characteristics of mixed sewage, with significant infiltrations from aquifers, so it is a highly diluted stream.

Some meaningful characterization parameters (expressed as mean values from daily analysis, in a "standard" month) of the 4 streams entering Tolmezzo WWTP are listed in tab. 2.1: from these data, the high acidity of condensate water stands out, together with the low solid matter and nutrient concentration of all P&P streams. As for COD load, the total plant load was estimated as 17,188 kg COD/day: whitening water accounts for 60% of this load, while condensate and paper WW contribute, respectively, for 24% and 10% of the total COD load. Municipal WW, finally, carries just the residual 6% of the load.

Another noticeable aspect is the high temperature of all P&P WW streams, in particular as for condensate and whitening water, that influence the kinetics of COD removal in the activated sludge process.

Table 2.1: Mean physicochemical and hydraulic characteristics of influent streams in Tolmezzo WWTP

Parameter	Whitening	Condensate	Paper	Urban
pH	6.8	2.9	7.1	7.9
T (°C)	24.1	33.3	21.4	
COD (mg/L)	846	3,566	156	214
SS (mg/L)	67	19	21	139
N_{tot} (mg N/L)	1.7	1.5	1.6	
P_{tot} (mg P/L)	0.3	0.4	0.2	
Q (m^3/h)	510	48	478	182
Load (kg COD/day)	10,355	4,108	1,790	935

2.2.3 UASB line in Tolmezzo WWTP

Condensate water, before being piped to Tolmezzo WWTP, is partially laminated in Mosaico P&P factory, through a 1,200 m^3 collection basin, and is sent to a stripping unit, to reduce sulphite concentration, that is very high in the stream. Then, conditioned WW is sent to the WWTP in the neutralization-pre acidification basins (total HRT of 3.5 h); here, the influent mixes with recirculated effluent from UASB unit, to partially laminate influent COD and increase alkalinity, reducing, at the same time, the required chemical dosage [35].

The first basin has V=50 m^3, and is provided of a stirrer, to homogenise influent and recirculated effluent characteristics, while the second basin (200 m^3 volume) allows chemicals dosage, both to correct pH (through soda) and to increase nutrients concentration (through ammonia and phosphoric acid). In addition, a control unit measures some fundamental parameters, such as T, sulphites, chlorides, pH, redox and TOC [35].

Conditioned wastewater is then pumped to UASB reactor, through 2 horizontal axis centrifugal pumps, characterized by 100 m^3/h maximum flow rate. UASB reactor (whose inner structure is visible in fig. 2.7) was projected for a HRT of 5.0 h, with a maximum up-flow velocity of 0.9 m/h; project OLR was 4.9 kg COD/m^3d (calculated on mean COD value) or 5.6 kg COD/m^3d (calculated on peak COD). The reactor is characterized by a modular geometry: there are 20 modules of 50 m^3 each, for a total volume of 1000 m^3 [35].

Biogas is separated from the effluent and the residual sludge granules in a three-phase separator, while condensation water is removed in a gravel filter.

Figure 2.7: Internal view of UASB reactor, installed in Tolmezzo WWTP

Specific biogas production from condensate water was assumed as 0.4 Nm^3/kg $COD_{removed}$. The gasometer, installed in biogas line, has a volume of 1000 m^3, allowing a 15 h storage. In case of emergency, biogas is burnt in a torch, which allows a maximum flow rate of 200 Nm^3/h. Biogas composition is assumed to be 80% CH_4, 19% CO_2, 0.5-1% H_2S: in order to protect CHP unit, biogas needs to be desulphurized, through an iron oxides packed bed. Finally, biogas is burned in CHP unit, that is characterized by an electric power of 115 kW_{el} [35].

UASB unit in Tolmezzo WWTP (fig. 2.7) was operating for some years (1995-2007), even if only a variable fraction (20-50%) of condensate water total flowrate (50 m^3/h, as reported also in tab. 2.1) was treated. The main operating problem, that was encountered in daily operations, was the high H_2S concentration in biogas, originated from anaerobic conversion of sulphate and sulphite, abundantly present in this stream, that caused odour emissions and required a high-efficiency biogas purification, in order not to damage the co-generative motor.

After 2007, technical and economic considerations of plant managing company led to the shut-off of UASB reactor; different societies followed one another in the last ten years in Tolmezzo plant management, and last IWS authority, CAFC, pushed for UASB section revamping, in order to reduce the organic load to the aerobic phase (and consequently excess sludge production), improving, at the same time, plant energy balance. In fact, as was shown in tab. 2.1, condensate water accounts, from an hydraulic point of view, for about 4% of the total flowrate, but, given its high COD concentration, the COD load fraction is as high as 24%.

It must be highlighted, finally, that in recent years condensate water composition (in terms of sulphur compounds) has significantly changed, because of the installation of stripping towers in Mosaico P&P plant: this enabled to reduce sulphate and sulphite concentration in the stream entering the plant, consequently reducing sulphur load to biogas line. However, a detailed analysis of sulphur compounds in condensate water will be presented in Chapter 5, where continuous pilot-UASB tests will be described.

2.3 UASB treatment: theoretical deepening

2.3.1 Anaerobic digestion principles

Generally speaking, anaerobic conversion of a generic organic substance proceeds through 4 successive phases (fig. 2.8), namely:

1. *Hydrolysis*;
2. *Acidogenesis*;
3. *Acetogenesis*;
4. *Methanogenesis*.

The final phase (methanogenesis) consists in two different reactions, namely acetoclastic (acetic acid-consuming) and hydrogenotrophic (H_2 and CO_2-consuming) methanogenesis; the reaction products basically consist in a mixture of CH_4 and CO_2, that is biogas, with other gases (O_2, N_2, H_2, H_2S) present as impurities [37].

As evident from fig. 2.8, differently from what happens in aerobic processes, a real trophic chain develops in anaerobic systems, where the products of one phase become reagents for the successive reaction [37]; process optimization is thus fundamental, because the slowing down of a single phase reduces overall reaction rate [38].

If compared to traditional aerobic treatment, anaerobic reactions are typically slower; moreover, a prevalence of energy production (catabolism) is observed over

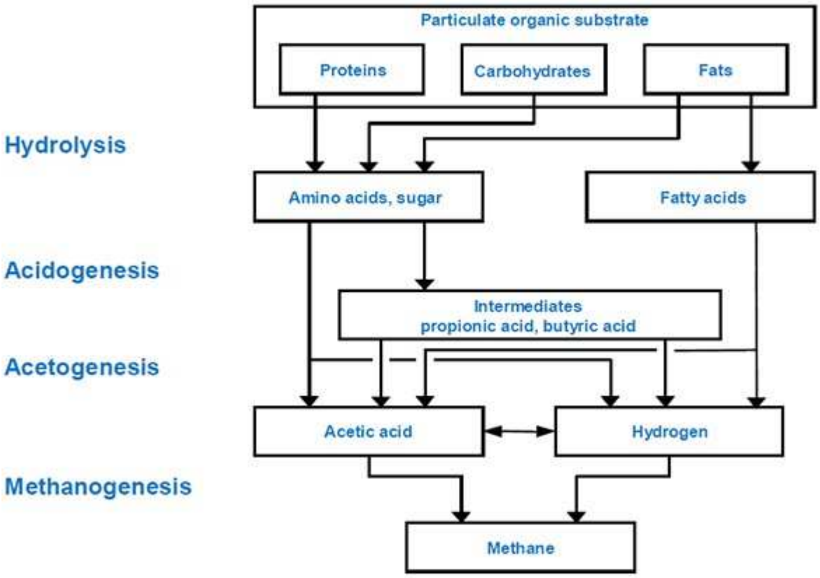

Figure 2.8: Anaerobic digestion process [36]

cellular synthesis (anabolism). Methanogenic bacteria are, among the different anaerobic microorganisms, the most sensitive to operating conditions (pH, T, OLR), and so, if process conditions are not properly controlled, they can be easily inhibited, reducing, or even stopping, methane production rate [37].

2.3.2 UASB reactor configuration

UASB (acronym of Up-flow Anaerobic Sludge Blanket) reactor belongs to the category of high-rate anaerobic reactors, and was introduced (as anaerobic filter) between the late 1960s and 1980. The main general feature of high-rate reactors is to carry out AD at a much faster rate than was possible earlier. All kinds of high-rate reactors, but in particular UASB, were used in the treatment of various types of biodegradable wastewaters, with considerable success [39].

The main advantages of UASB technology can be summarized as follows:

- Substantial compactness (low required volumes);
- Low operational cost;
- Energy recovery (through biogas);
- Low sludge production, particularly significant if compared to flocculent processes.

UASB reactors demonstrated their efficacy in the treatment of high-strength industrial wastewaters, containing easily hydrolisable substrates, such as sugar industry wastes, distillery wastes and brewery wastes [40]. The performances of UASB reactor in treating difficult-to-hydrolize and complex substrates, such as effluents from food and milk processing plants, or slaughterhouse WW, have been less satisfactory [41], but continuous improvement of UASB design, start-up and operation have solved this shortcomings to a large extent [42].

A typical scheme of UASB reactor is reported in fig. 2.9. Wastewater is fed at the base of the reactor and flows upwards; the influent passes through granular sludge bed, that is maintained as suspended, and anaerobic reactions take place. Successively, wastewater crosses blanket zone, that is characterized by a lower biomass density, where sludge particles are separated from biogas bubbles, that move upwards, together with the effluent. Biogas is finally separated from the effluent and residual solid particles in the upper part of the reactor, by means of a three-phase separator [37].

Granular biomass, differently from classical flocculent biomass, has high density and optimum sedimentability. An ideal granular sludge (fig. 2.10) can

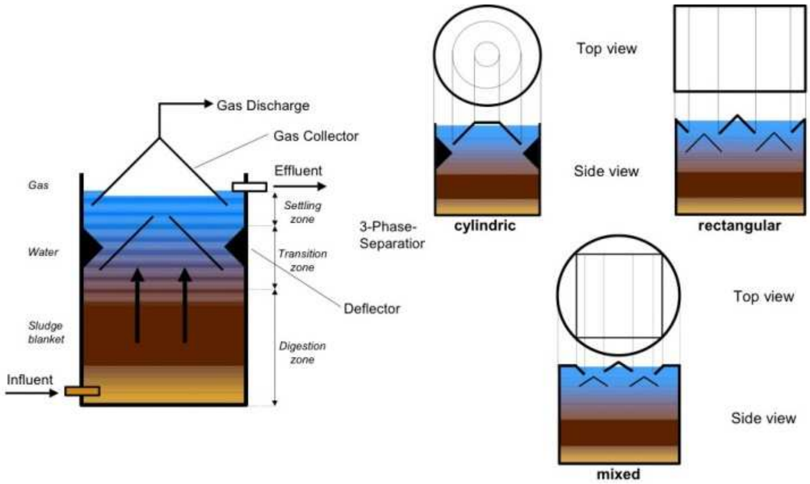

Figure 2.9: UASB reactor scheme [43]

be viewed as a layered structure, where methanogenic bacteria occupy the inner part, attached to an inert nucleus, while hydrolytic and acidogenic bacteria are positioned in an outer layer [39]. Granule diameter is in the range of 0.1-5 mm, and SVI index is typically less than 20 mL/g SS (consistently lower than classical flocculent sludge).

Due to the optimum biomass immobilization in the system (that consequently increases SRT), UASB uncouples biomass retention and liquid retention [45]; moreover, being characterized by high SRT/HRT ratio, UASB allows to reduce HRT, to values even lower than 10 h, if the influent WW is highly biodegradable. However, high velocity reactors, such as UASB, are mostly indicated for dissolved organic compounds treatment, rather than suspended solids removal, because the latter require longer HRT, to be hydrolysed. Excess sludge production is negligible, if compared to aerobic units, and this aspect is noticeable, because sludge treatment and disposal is one of the main operating costs in WWTPs.

Typical UASB operating parameters, as reported in literature, are the following [37]:

- Influent COD concentration: 5-10 g/L;
- Operating temperature: 32-36 °C;

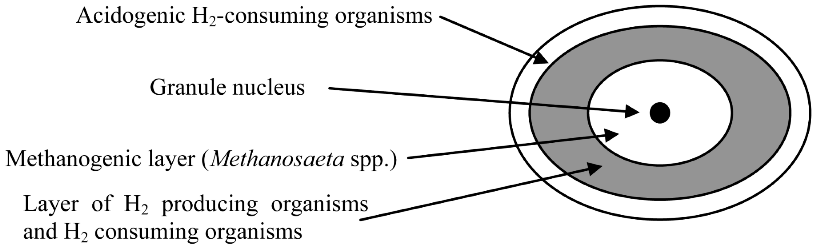

Figure 2.10: UASB granule inner structure [44]

- OLR: 12-20 kg COD/m^3d;
- Up-flow velocity<1.2 m/h (higher up-flow velocity is used in start-up, to wash-out low-density granules, selecting only high-density particles);
- HRT>6 h;
- COD removal: 75-90%;
- Reactor height: 4-7 m.

UASB reactor start-up is critical, and needs to be sustained through granular sludge inoculum, until 10-30% of the total reactor volume [37].

2.3.3 UASB applications

Industrial WW is a major source of pollution, due to its high organic content, and is characterized by an extreme variability in COD concentration (1-200 g/L) [46]. A major factor for biological treatment is substrate biodegradability, generally expressed through BOD/COD ratio: WW is considered biodegradable if BOD/COD is higher than 0.5.

Conventional biological systems usually fail to effectively treat high-strength WW, and produce low-quality effluents. Thus, it is preferable to anaerobically treat this high-loaded WW; in particular, UASB reactor can be an interesting option, because it allows granular sludge formation, without any attachment media (differently from anaerobic filters). It offers a dense and strong microbial structure, with good settling ability, high biomass retention, tolerance to toxicity and resistance to shocks [47]. The three-phase separator maximizes biomass retention in the reactor, without the need for an external clarifier [48]. Actually, UASB and its variant, EGSB, account for 72% of anaerobic reactors present all over the world [49]. The main downside of this technology, as mentioned above, is the long start-up period, accompanied with significant sludge washout.

UASB performances are negatively affected by low temperature operations, where the hydrolysis of the entrapped COD becomes limited, resulting in solids accumulation in the sludge bed, especially when working at high organic loadings [50]. Low T, high loading rate and high TSS result in a shorter SRT, decreasing biogas production and COD removal efficiency. Moreover, it should be reminded that only a change in nitrogen and phosphorous chemical forms takes place, so nutrient removal is not significant [51].

Given these general aspects, it can be inferred that UASB reactor can be useful as pre-treatment of high-strength wastewater; however, it is usually followed by an aerobic polishing step. In fact, anaerobically treated effluents contain solubilized organic matter, optimum for subsequent aerobic treatment, due to the reduced organic content and enhanced amount of nutrients [52]. Moreover, complete stabilization of high-strength organic matter cannot be achieved anaerobically, and this results in an effluent quality that fails to comply with the legislative standards [53] (in Italy, the main reference for WWTP effluents is D. Lgs. 152/2006). The advantages of this combined process (anaerobic + aerobic) can be summarized in great potential for energy recovery, high overall treatment efficiency, less sludge amounts to dispose, low energy consumption and minimum volatilization in the aerobic phase.

2.3.4 UASB operational parameters

Organic Loading Rate (OLR) is an important parameter, significantly affecting microbial ecology and performance of UASB systems. OLR integrates reactor characteristics, operational characteristics, and bacterial mass and activity [54]; moreover, it is related to COD concentration and HRT, thus a good balance between these two parameters needs to be found, for good digester operations [55]. In fact, excessive OLRs reduce COD removal efficiency; on the other hand, gas production tends to increase with OLR, until a point where methanogens could not work quick enough to convert acetic acid to methane [55].

pH is another fundamental parameter: methanogenic bacteria activity is strongly affected by pH. The optimum pH range for microbial activity is 6.8-7.2, while pH values lower than 4, or higher than 9.5, are not tolerable [57]. In literature, several cases of reactor failure have been reported, caused by accumulation of Volatile Fatty Acids (VFA), that caused pH drop and consequently

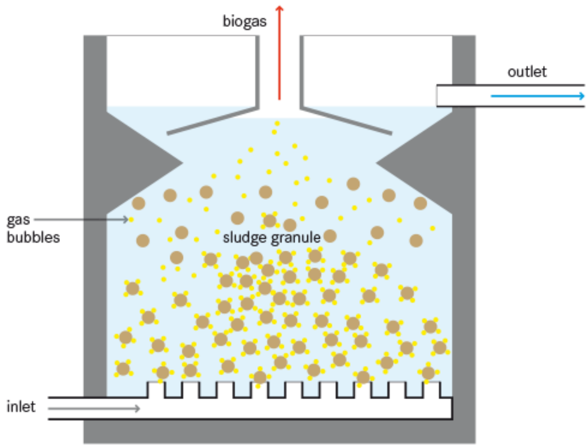

Figure 2.11: Another UASB reactor scheme [56]

inhibited methanogenesis [58]. Due to this fact, VFA concentration, being also an intermediate product of AD process, is an important parameter to monitor, in order to guarantee good reactor performance [59].

Alkalinity helps to give a buffering capacity to UASB reactor, providing a hedge against sharp changes in pH [39]. For UASB reactor, an optimum alkalinity range of 250-950 mg/L was suggested in literature [60].

Up-flow velocity, v_{up}, is another meaningful parameter, because it maintains the mixing and HRT of substrate and biomass. Many researchers reported a permissible v_{up} of 0.5-1.5 m/h [55], even if values greater than 1 m/h can cause granules disintegration, due to increasing shear stress, and the resulting fragments may wash-out the reactor [61].

In addition, temperature, T, is a significant factor, that directly influences biomass activity. The influence of T on microbial growth and biodegradation rate can be described by the Arrhenius equation [62], where the rate constant of a generic reaction is related to T, activation energy (E_a) and pre-exponential factor, A.

$$k = Ae^{(-E_a/RT)} \tag{2.1}$$

It has been proved that anaerobic bacteria show good activity in the mesophilic range (T= 30-40 °C). Operation of anaerobic reactors under thermophilic (40-55 °C) conditions offers a number of advantages, such as increased reaction rates and improved biodegradability of organic compounds. However, start-up and operation of a thermophilic reactor is cumbersome, due to the high sensitivity of thermophilic microorganisms to variations in OLR, influent composition and reactor pH [55].

Nutrient request (in terms of N and P) is generally limited in UASB reactors, and is typically calculated on classical COD:N:P ratio of 350:5:1 [37]. Methanogens utilize ammonia as a source of nitrogen, and even ammonium-rich substrates can be successfully treated in UASB reactors, if a sufficient acclimation period is forecast [63].

Finally, inhibitory compounds concentration must be carefully monitored, to avoid negative effects on biomass: the main compounds that are typically considered as toxic are NH_3, that comes from proteins degradation, H_2S, that originates from sulphate reduction (operated by Sulphate Reducing Bacteria, SRB), and heavy metals [64]. Successful bacteria acclimation, however, can allow to operate under even high concentrations of NH_3 (in the order of g N/L).

2.4 UASB modelling

A growing importance is being given nowadays to process modelling, that is a useful tool to assist project engineers in design of new WWTPs, as well as in revamping of existing plants. Modelling helps to study plant performances under different operating conditions, without doing full-scale tests, that are anti-economic and, in addition, can create disturbances to the process itself.

As for AD processes, given their inner complexity, it is difficult to evaluate the impact of all process variables on digesters performance. Hence, it is not trivial to optimize the design and operation of these plants. Pilot testing, also as experienced in this research work (Chapter 5), is challenging, due to the extended time period that is required. The use of models for predicting process performance over a range of design and operating conditions becomes therefore attractive [66].

As for UASB process, the first proposed approach is to use ADM1 model, that has been developed by International Water Association (IWA) [67], even if this model has been specifically designed for solid-treating anaerobic digesters, rather than liquid-treating reactors, such as UASB. There exist also specific UASB models; some of them will be briefly described in the following. However, it should be reminded that more complex models require a higher number of input parameters, so the choice of the model to apply should consider also the capability to collect detailed data about biomass and substrate characteristics, as well as kinetics and stoichiometry.

2.4.1 ADM1

Over the years, a range of models were created for AD processes; in 2002 there was a move by IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes to develop a common model, that could be used by researchers and practitioners [67]. The developed model has a structure that is similar to the IWA Activated Sludge Models (ASM1, ASM2, ASM3), that have received acceptance by practitioners over the last 20 years.

The ADM1 model is a structured model, that reflects the major processes involved in the conversion of complex organic substrates into methane, carbon dioxide and inert byproducts [66].

This model includes disintegration of complex solids into inert substances, carbohydrates, proteins and fats; its structure is schematically represented in fig. 2.12. The disintegration products are respectively hydrolyzed to sugars, amino acids and long chain fatty acids (LCFA). Carbohydrates and proteins are fermented, to produce volatile organic acids (acidogenesis) and molecular hydrogen. LCFA are anaerobically oxidized to produce acetate and molecular hydrogen. Propionate, butyrate and valerate are then converted to acetate (acetogenesis) and molecular hydrogen. Methane is produced by both cleavage of acetate to methane (aceticlastic methanogenesis) and reduction of carbon dioxide by molecular hydrogen (hydrogenotrophic methanogenesis) [66].

The model employs state variables to describe the behaviour of soluble (S) and particulate (X) components. All organic species and molecular hydrogen are described in terms of COD. Nitrogenous species and inorganic carbon species are described in terms of their molar concentrations.

Soluble components (S) are those able to pass through microbial cellular walls, and include the monomers of complex polymers (sugars, amino acids, long chain fatty acids), volatile organic acids (propionate, butyrate, valerate, acetate), hydrogen, and methane. In addition to organic species, the model considers inorganic carbon (carbon dioxide and bicarbonate) and nitrogenous species (ammonia and ammonium) [66].

All of the species that dissociate as a function of pH (VFAs and ammonia) have variables defined for both the protonated and non-protonated species. The model maintains a charge balance among ionic species, and hence there are variables for inorganic anions and cations, including the hydrogen ion. The

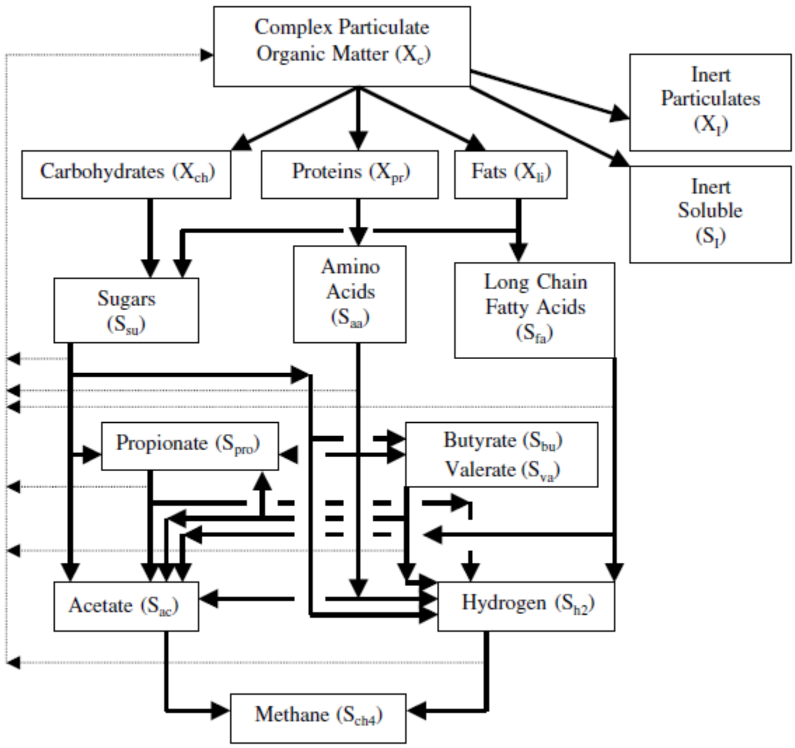

Figure 2.12: Conceptual AD scheme for ADM1 model [66]

model solves for the hydrogen ion concentration, and thereby pH, by ensuring chemical neutrality in solution [66].

Particulate species (X), instead, consist of either active biomass or particulate substances, that are incapable of directly passing through bacterial cell walls. The microbial species that are considered in the model include sugar fermenters, amino acid fermenters, LCFA oxidizers, butyrate and valerate oxidizers, propionate oxidizers, aceticlastic methanogens and hydrogenotrophic methanogens. Non-microbial particulate species include complex organics, that either enter the process in the influent, or that result from the death and decay of microbial species and the products of disintegration of the complex organics. This latter group consists of carbohydrates, proteins and LCFAs [66].

Substrate conversion processes are described by a number of kinetic expressions, that model the conversion rates in terms of substrate concentrations and rate constants. The disintegration of complex particulate organic matter (X_c) and hydrolysis of carbohydrates (X_{ch}), proteins (X_{pr}) and fats (X_{li}) are described by first order rate expressions. Substrate conversion processes have Monod-type kinetic expressions, while endogenous decay processes are first order in biomass concentration [66].

For each of the above-mentioned processes, the rate of product generation is related to the process rate through stoichiometric coefficients. For example, the rate of organism growth is related to the rate of substrate consumption through the yield coefficient for the organism on the substrate. This format is consistent with the approach that is employed in the ASM models [66].

In the model, all microbially mediated substrate conversion processes are subject to inhibition by extremes of pH. All anaerobic oxidation processes are subject to inhibition by accumulation of molecular hydrogen, and aceticlastic methanogenesis is inhibited at elevated free ammonia concentrations. Liquid–gas mass transfer of gaseous components (methane, carbon dioxide and molecular hydrogen) is described by mass transfer relationships. Hence the application of the model equations requires separate mass balances for the liquid and gas phases of the components [66].

2.4.2 Specific UASB models

ADM1 model has been used to simulate UASB reactor performance at different scales, for treating a large WW variety. However, in these studies the flow pattern of UASB was assumed to be near-ideal mixing, because ADM1 was initially developed for continuously stirred-tank reactor (CSTR) systems [68].

The hydrodynamic characteristics of UASB reactors have been extensively explored under different conditions. Batstone et al. [69] indicated that the flow pattern in a UASB could be between plug flow and ideal mixing flow, instead of ideal mixing. Some literature papers pointed out that the hydrodynamics of UASB reactors could be described by a dispersive model, with a proper diffusion coefficient [70], whereas others showed that the multi-CSTR model could be effectively employed to simulate UASB reactor hydrodynamics [71].

These results imply that the flow pattern of UASB reactors is very important but complex, and that therefore it cannot be simply regarded as ideal mixing. Up to now, very limited work has been done to consider complex reactor hydrodynamic characteristics simultaneously, when developing an ADM1-based model to describe UASB reactors.

Axial dispersion model

In the work by Chen et al. [72], an axial dispersion model was developed to simulate the axial soluble component and biomass species distributions in a UASB reactor. The hydrodynamic transport of the components was described using the following equation, referred to the general scheme reported in fig. 2.13:

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial z}(D_1 \frac{\partial C}{\partial z} - u \frac{\partial C}{\partial z}) \tag{2.2}$$

The boundary conditions were the Danckwerts equations [72]:

$$|D_1 \frac{\partial C}{\partial z}|_{z=0} = u(C_l - C_0) \tag{2.3}$$

$$|\frac{\partial C}{\partial z}|_{z=H} = 0 \tag{2.4}$$

Combining these equations with the bio-kinetic part, the distributions of the soluble substrate and insoluble biomass in the ADM1-based dispersive model were obtained as follows [72]:

$$\frac{\partial S_i}{\partial t} = \frac{\partial}{\partial z}(D_1 \frac{\partial S_i}{\partial z}) - u \frac{\partial S_i}{\partial z} + r_{S,i}(z,t) \tag{2.5}$$

$$\frac{\partial X_i}{\partial t} = \frac{\partial}{\partial z}(D_2 \frac{\partial X_i}{\partial z}) - u \frac{\partial X_i}{\partial z} + r_{X,i}(z,t) \tag{2.6}$$

In eq. 2.5 and 2.6, the right-hand side represents the sum of the dispersive and convective components, with the bio-dynamic term ($\mathrm{r_{S,i}}$ and $\mathrm{r_{X,i}}$), indicating reaction of the substrate (S) or biomass (X). A similar distribution of soluble

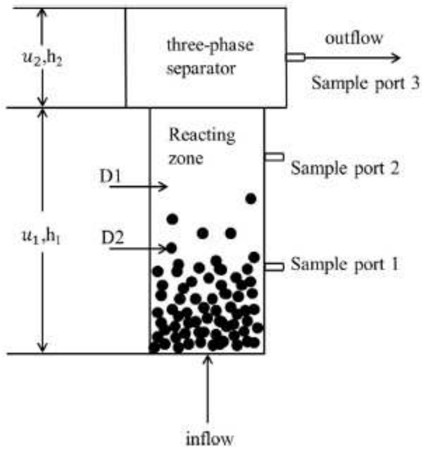

Figure 2.13: Scheme of UASB reactor using the ADM1-based dispersive model [72]

and insoluble compounds was adopted, but a smaller conversion coefficient, D_2, was introduced for the insoluble terms. Moreover, in this model two different up-flow velocities were used, with a smaller one, u_2, in the upper separator, due to a greater cross sectional area; a boundary was assumed, as can be seen in fig. 2.13, between the reaction zone and the three-phase separator. It was assumed that, for $0<s<h_1$, $u=u_1$, and, for $h_1<s<h_1+h_2$, $u=u_2$ [72].

The results of the model implementation, reported in [72], showed that the dispersive model fitted best the results, if compared to a multi-CSTR model.

AM2 model

The AM2 model was proposed by Bernard et al. [73], as a simple two-step (acidogenesis-methanisation) mass balance model. In the first step, acidogenic bacteria, X_1, consume the organic substrate, S_1, producing VFA (S_2), CO_2 and more bacteria. Methanogenic bacteria, then, consume VFA, originating CH_4, CO_2 and more microorganisms. The two biological reactions can be written as follows [74]:

$$k_1S_1 \rightarrow X_1 + k_2S_2 + k_4CO_2 \tag{2.7}$$

$$k_3S_2 \rightarrow X_2 + k_5CO_2 + k_6CH_4 \tag{2.8}$$

The specific reaction rates are:

$$r_1 = \mu_1 X_1 \tag{2.9}$$

$$r_2 = \mu_2 X_2 \tag{2.10}$$

The other state variable that is considered in the model is the inorganic C. It is assumed that inorganic C is formed by CO_2 and bicarbonate, while total alkalinity is the sum of bicarbonate alkalinity and VFA. In the liquid phase, CSTR behaviour is assumed; moreover, an α parameter is introduced, representing the solid fraction that exits the reactor, to incorporate the effects of solid retention in the reactor. The equations of the dynamic model can be written as follows, where D is the dilution rate (in d^{-1}) and q_C is the CO_2 flux.

$$\frac{dX_1}{dt} = [\mu_1(\xi) - \alpha D]X_1 \tag{2.11}$$

$$\frac{dX_2}{dt} = [\mu_2(\xi) - \alpha D]X_2 \tag{2.12}$$

$$\frac{dS_1}{dt} = D(S_{1,in} - S_1) - k_1\mu_1 X_1 \tag{2.13}$$

$$\frac{dS_2}{dt} = D(S_{2,in} - S_2) + k_2\mu_1 X_1 - k_3\mu_2 X_2 \tag{2.14}$$

$$\frac{dC}{dt} = D(C_{in} - C) - q_C + k_4\mu_1 X_1 + k_5\mu_2 X_2 \tag{2.15}$$

Methane flux, q_M, can be finally evaluated as follows:

$$q_M = k_6\mu_2 X_2 \tag{2.16}$$

As already discussed, the main problem with dynamic models is their complexity: many parameters are not easy to measure experimentally [75]. Simple models, on the other hand, such as AM2 model, omit some important processes, such as mass transport resistance [76]. So, as previously discussed, the right balance between model accuracy and ease should be found, depending on the specific purposes. This simple model, anyway, can be a first approach for UASB modelling, requiring a limited number of input data.

Multi-CSTR method

In the work by Rodriguez et al. [71], the multi-CSTR method was used to simulate a UASB reactor; the number of CSTRs in which the UASB was divided, N, was related to the Peclet number (Pe), that indicates the relative ratio between advective and diffusive transport rate:

$$N = \frac{Pe}{2} + 1 \tag{2.17}$$

A quasi-steady state mass balance for substrate concentration in the granule was applied, because it was assumed that the amount of substrate degraded in a time interval was much greater than the substrate variation in the granule, in the same time interval. In eq. 2.18, D_A is the substrate diffusion coefficient within the granule, while S_p is substrate concentration within the granule [71].

$$D_A \frac{1}{r^2} \frac{d}{dr} (r^2 \frac{dS_p}{dr}) = -r_s \tag{2.18}$$

The kinetic term was modelled with Monod equation [71]:

$$r_s = -\frac{\mu_{max}}{Y} \frac{X}{K_s + S_p} S_p \tag{2.19}$$

Three governing equations were used, describing, for each small reactor, the concentration of substrate (S_i), active biomass (X_i) and inactive biomass (E_i).

$$\frac{dS_i}{dt} = \frac{Q}{V_i}(S_{i-1} - S_i) - r_i \tag{2.20}$$

$$\frac{dX_i}{dt} = r_i Y - k_d X_i \tag{2.21}$$

$$\frac{dE_i}{dt} = k_d X_i \tag{2.22}$$

The reaction term, r_i, was expected to change in every time interval, so a new kinetic value was continuously calculated, using the following equation, where q represents the flux (expressed as kg/m^{2}h) and N_p is the number of granules per volume of the reactor (granule/m^3):

$$r_i = q\, 4\pi r^2 N_p \tag{2.23}$$

This model can be used as another approach to model UASB systems, and the effective model to apply should be evaluated case by case, by studying the best fitting between experimental and modelled data.

2.5 Conclusions

The case-study analysed in this Ph.D. research, Tolmezzo WWTP (143,000 PE), was introduced in this chapter, mainly focusing on plant process scheme (traditional activated sludge, followed by tertiary physicochemical coagulation-flocculation) and meaningful characteristics of the influent streams (three P&P WW streams and a municipal WW stream). A particular attention was put on UASB line, being this technology one of the main targets of this work.

Moreover, it was seen in this chapter that anaerobic processes, and in particular UASB, can be a good practice for the treatment of high-strength industrial wastewater, and the most important operating parameters were briefly discussed. In addition, a theoretical deepening was made, to describe UASB process modelling. Actually, given the impossibility of collecting a detailed spectrum of data for modelling, it was not possible to effectively model the results of the BMP and UASB tests, a part for a simple regression analysis, that was performed on BMP tests (Chapter 4). This further deepening can be suggested as a possible prosecution or implementation of the actual work.

Chapter 3

Characterization of selected substrates

3.1 Introduction

In the first part of this chapter, a brief literature overview will be presented, to introduce the physicochemical characteristics and the current technologies applied to treat the selected substrates: CW, Pulp & Paper WW, OFMSW leachate, brewery waste, slaughterhouse waste. Sonication will then be described, as an interesting pre-treatment, both to increase energy recovery from AD (through disaggregation of large molecules), and to facilitate resource recovery from ultra-filtration processes (described in detail in Chapter 6).

Successively, the obtained physicochemical characterization results will be presented and discussed with literature evidences, preceded by Material and Methods section, where analytical methods will be mentioned. A particular focus will be put on macromolecular composition, because it directly influences the behaviour of anaerobic reactors: proteins, if present in high concentrations, can even lead to reactor failure, due to excess NH_3 formation, while lipids, if not efficiently degraded, can form foam and greases, which accumulate in the system. Elemental analysis results will be presented as well: they were particularly useful to estimate C/N ratio from selected matrices.

The results from laboratory analysis are mandatory to correctly plan Biochemical Methane Potential (BMP) tests, that will be reported in Chapter 4, and continuous UASB tests, that will be presented in Chapter 5.

Figure 3.1: Cheese whey (main sub-product of cheese production chain) [82]

3.2 Literature evidences

3.2.1 Cheese whey

Dairy industry is one of the main sources of industrial effluents in Europe [77]; dairy WW characteristics are highly variable, depending on the specific final products and operation methods used in the manufacturing plant [78].

In fact, cheese effluents have a broad COD concentration range of 0.8-102 g/L (BOD=0.6-60 g/L), and biodegradability index, expressed as BOD/COD ratio, is 0.4-0.8 [79]. The main organic pollutants founded in this stream are lactose and fats [80]; a wide pH range has been reported in literature (3.3-9.0), even if this stream is typically acidic. SS, TKN and TP literature values are respectively in the range of 0.1-22.0 g/L, 0.01-1.7 g/L and 0.006-0.5 g/L [79]; cheese effluent composition can be approximated to a C:N:P ratio of 200:3.5:1, that is N deficient for aerobic or anaerobic processes [81].

The typical manufacturing process in dairies can be summarized as follows: the milk is stored at low temperature in stainless steel tanks, and is then sent to coagulation, where microbial or vegetable rennet is added. After the required time, the fermented milk produces the curd, that is cut and converted into the desired commercial products. Part of Cheese Whey (CW), the so called *first whey*, resulting from hard cheese production, is processed again, obtaining cottage cheese or curd cheese [83]. These further processing leads to the generation of *second whey*. So, cheese manufacturing industry produces three different WW streams, namely first whey, second whey and CW wastewater (coming from pipelines, storage and tanks washing) [79]. Given the importance of separately characterizing the dairy streams, both first and second whey will be analysed in this work.

CW (fig. 3.1) is the most polluted stream produced by dairy industry, because of its extreme organic load (BOD= 27-60 g/L; COD= 50-102 g/L); BOD/COD ratio is normally above 0.5, indicating a easily biodegradable substrate [81]. Another noticeable characteristic of CW is the low buffering capacity, that is responsible for the rapid acidification, frequently observed in biological treatments [84]. Casein precipitation leads to the formation of two different kinds of whey, namely acidic (pH<5) and sweet (pH=6-7) whey; typically, acidic whey has a higher ash and lower protein content than sweet whey [79].

Second CW contains about 60% of dry matter content of first whey [85], but it retains a significant COD concentration (up to 80 g/L) and a high salinity, even higher than first whey, due to further salts addition in processing [79]. COD, BOD and TSS are lower than first whey, because of the flocculation process, while pH is generally acidic and biodegradability ratio is close to 0.5 [79].

Finally, cheese whey WW has a significantly lower contamination than CW, even if its pollution is variable, depending on the quantity of whey eventually discharged with washing water [79].

From a massive point of view, it is important to highlight that, for the production of 1 kg of cheese, 10 kg of raw milk are needed, and 9 kg of CW are generated; so, high amounts of whey need to be properly managed and treated, even by small dairies [81]. According to the physicochemical characteristics previously described, whey, if improperly managed and discharged, can cause an excess of oxygen consumption, impermeabilization, eutrophication and toxicity in receiving environments [81].

Because of the high organic content of CW, alternative treatment techniques have been developed and applied, including [86]:

- Land application as fertilizer;
- Valorisation through biological treatments;
- Physicochemical treatments, to produce and recover valuable compounds, such as proteins and lactose.

Physicochemical treatments have shown to be successful for dairy companies with high processing volumes and enough capital to invest in their implementation.

Figure 3.2: Pulp and paper mill [90]

On the contrary, for Small to Medium Enterprises (SMEs), such as the plants studied in this work, CW disposal is challenging, because they do not have the economic resources required for the proper treatment and valorisation. Therefore, these companies prefer to give away this residue for farm animal feeding [81]; this is actually done also by the dairies located in the mountain area of Friuli-Venezia Giulia region, that represent the case-study.

Anaerobic digestion (AD) of this high-loaded matrix can be a triple action process for CW treatment, also for SMEs, if simple reactor configuration is preferred: pollution discharge reduction, energy obtainment, and nutrient recovery [87].

3.2.2 Pulp & Paper wastewater

Pulp and Paper (P&P) industry (fig. 3.2) generates relatively large amounts of both WW and solid waste [88], even if the effective amounts produced by each plant depend on the raw materials used for production (i.e. wood or non-wood matrices, recovered fibers). After the separation of stock material, the main processing steps include pulping, bleaching and, in the end, paper making [89]. P&P mill is a relatively high water-dependent industry, if compared to other industrial sectors; water is generally withdrawn from surface and ground waters, is used for most of the process stages and forms the main liquid reject from the industry [89].

Due to global concerns on water resources scarcity, though environmental regulations have been developed, to ensure the sustainable water use for industrial users. Indeed, there was a strong push for water consumption reduction in P&P industry, that was enormous at the beginning of 20th century (200-1,000 m^3/ton paper). For example, nowadays a German P&P industry has succeeded in reducing water consumption to values as low as 13 m^3/ton paper [91].

Moreover, recovered paper volume greatly increased in recent decades, leading to a further decrease in P&P WW, because recovered fibre mills are less water intensive than virgin fibre P&P mills [92]. Actually, only a small part of the water is consumed throughout the process: for instance, in US 88% of intake water is returned back to surface waters, after being treated, while only 11% is evaporated and 1% is embedded in the product or solid waste [93]. A simplified process scheme for P&P production from virgin fibres is reported in fig. 3.3. As for P&P WW characteristics, it must be highlighted that several chemical additives are used in the process and, if chemical recovery is not applied, they exit the plant in the WW [89]. Moreover, the presence of toxic and non-toxic compounds, such as resin acids, sterols, waxes and esters is sometimes detected in WW [94].

Various techniques applied for P&P WW treatment have shown different capabilities to remove pollutants. Physicochemical methods showed acceptable performances on pollutants removal, and, in fact, they are applied also in Tolmezzo WWTP, as a tertiary treatment of WW, but, on the other hand, they are very expensive (due to the huge chemicals cost). Biological methods are efficiently used for the treatment of WW from many types of P&P production processes, even if it should be considered their limited effect on the refractory fraction, significantly present in this WW (being rich in lignin).

Although activated sludge processes are currently the major treatment for P&P mill effluents (as previously described, they are in fact the main treatment applied also in Tolmezzo WWTP), AD has attracted a great amount of attention in recent years, due to its inherent merits, such as biogas production and solid waste minimization, which have made it an attractive candidate for P&P WW treatment [89].

In addition, the treatment of P&P WW normally produces a large amount of primary and secondary waste sludge, whose management and disposal are contributing to about 60% of the total P&P WW treatment cost [95]; however, actually a pre-treatment is performed in Tolmezzo P&P factory, so as to remove most of the solid content, before WW streams enter the WWTP. In fact, as reported also in Chapter 2, a low SS concentration is typically measured in all the influent P&P streams.

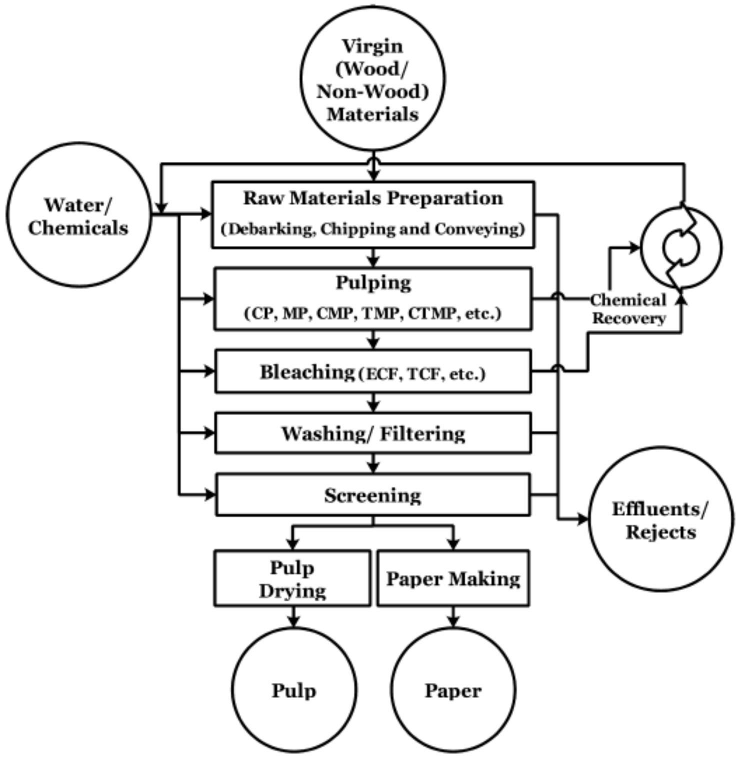

Figure 3.3: Scheme of P&P production process from virgin fibres [89]

Figure 3.4: Organic Fraction of Municipal Solid Waste (OFMSW) [97]

3.2.3 OFMSW leachate

Organic Fraction of Municipal Solid Waste, OFMSW (fig. 3.4), essentially consists of food and garden waste from domestic, commercial and street cleanings. It is the main cause of smell and nuisance in municipal solid waste (MSW), and is responsible for most of the environmental hazards associated with MSW management, such as formation of polluting leachate and methane gas, under anaerobic conditions [96]. Nowadays, OFMSW, separately collected from household and commercial facilities, is often anaerobically digested, either in mono-digestion or in co-digestion with other substrates, such as sewage sludge; to this purpose, several mature commercial processes are available, including BTA, Dranco and Valorga [98]. However, OFMSW is a complex and heterogeneous material, characterized by a TS content of 20-30% [99], and many questions still remain about the most effective AD process to apply [100].

Water leaching has been used in scientific literature to extract soluble organic compounds from OFMSW; the leachate is readily available for microorganisms, if compared to lignocellulosic substances, such as the ones present in garden waste. It has been shown that, by extracting the soluble substances from OFMSW (resulting in leachate and bagasse, that is the residual solid fraction), it is possible to obtain the same amount of biogas in less time than with wet or dry conventional technologies [101].

Innovative solutions to treat OFMSW include the use of Leach Bed Reactors (LBR), that allow OFMSW digestion in static piles, with leachate recirculation over the fermenting material. This technology can be applied in high solids materials (25-50% TS) treatment; in addition, it has the advantages of simplicity (little moving parts) and reduced heat demand [102]. In literature, the coupling of a solid-treating LBR and a liquid-treating UASB has been named Hybrid Solid-Liquid Reactor (HASL) [103]. The main operating problem in these reactors is LBR clogging, due to high waste density; bulking agents can be used, to avoid clogging and facilitate leachate percolation [104]. Two-phase digestion, in this configuration, can be a good solution: water is sprayed over the top of waste pile, and percolates, assimilating soluble fermentation products, such as organic acids, leading to COD increase in the liquid phase [96]. Leachate is then transferred to UASB reactor, to produce methane.

An alternative to percolation is mechanical solid-liquid separation through mechanical pressing (such as use of screw presses), that can be used to reduce OFMSW moisture content, without adding any process water to the waste; for example, in the work by Nayono et al. [105], a mash-separator was employed to this purpose. From 1 t of OFMSW, 700 kg of solid material for composting, and 300 kg of so-called *press water*, with a high organic content for AD, were generated. Press water showed a tCOD of 213 g/L, while sCOD was as high as 100 g/L; its maximum CH_4 production was estimated as 0.49 m^3 CH_4/kg VS [105].

It must be underlined, in any case, that, after extraction of soluble compounds from waste, the residual solid fraction needs to be properly managed, typically with a composting process, in order to stabilize the organic matter and to eliminate pathogens and unwanted materials. This process, if applied locally, can contribute to the closure of organic waste cycle, leading to an effective application of circular economy principles (previously described in Chapter 1).

3.2.4 Brewery waste

In the food industry, the brewing sector (fig. 3.5) holds a strategic position, with annual world beer production exceeding 1.34 billion hL in 2002 [106]. The brewing process is energy intensive and uses large volumes of water; beer production includes the blending of malt, hops and sugar extracts with water, followed by its subsequent fermentation with yeast [107]. A number of batch-type operations are employed in processing raw materials to the final beer product.

Water is a substantial ingredient of beer, composing 90-95% of the final product by mass [109]. Water chemistry can influence not only beer taste, but

Figure 3.5: Brewery [108]

also the brewing efficiency; water consumption for modern breweries generally ranges from 0.4 to 1 m^3/hL of produced beer [110].

Brewing residues include brewery wastewater and solid waste, that is composed of hops, trub, sludge, surplus yeast [109]. Brewery spent grain (BSG), fig. 3.6, in particular, represents the main brewery organic residue, and has a significant commercial value, because it can be sold as by-product for livestock feed; it is generally characterized by a water content of 80% [109]. Another process residue is whirlpool residue, that originates from a separation process, aimed at separating hop pellets and trub from wort, after wort boil. The wort is pumped into a whirlpool vessel at high velocity for 10-20 min; the wort starts spinning itself and allows separation of wort from residual pile [111].

Surplus yeast (fig. 3.7), instead, is recovered by natural sedimentation at the end of fermentation and maturation process, but only a fraction can be reused, while spent yeast can be sent to animal industry as feeding supplement, given its high content of proteins and B vitamins [109]. Two main types of yeast are used in brewing industry, depending on its separation: top-fermenting (whose strain is *Saccharomyces cerevisiae*) and bottom-fermenting (whose strain is *Saccharomyces uvarum*). Darker beer (such as IPA) results when using top-fermenting yeast, while a clear beer (such as lager) originates when choosing bottom-fermenting yeast [117].

Figure 3.6: Brewery Spent Grain (BSG) [116]

Brewery wastewater, being highly biodegradable, can be successfully treated using UASB technology [112]; surplus yeast, as well, has been successfully digested also at full-scale [113]. However, BSG is the main by-product generated during beer production, and its worldwide annual production has been estimated as 38.6 x 10^6 tons [114]. As for BSG, many different possibilities are feasible; among them, use as food supplement and cattle feed are the most widely applied [115]. Actually, as observed also in the case of dairies, selected breweries in Friuli-Venezia Giulia region, having small-to-medium size, usually employ their BSG as cattle feed.

Only in recent years BSG was considered as an energy substrate; earlier studies revealed that BSG has a biogas potential, even if conventional AD processes are economically unattractive, because of long HRTs and slow biodegradability [118]. In fact, BSG is composed of cellulose (16.8-25.4%), hemicellulose (21.8-28.4%), lignin (11.9-27.8%), protein (15.2-24.0%), lipid (up to 10%) and ash (2.4-4.6%) [119], so a significant fraction with low biodegradability is present. Furthermore, the high protein content (C/N=3-5; TN=11-13 g/kg of wet weight) can lead to NH_3 inhibition, if BSG is used as mono-substrate in AD. Therefore, substrate dilution or co-substrate addition (using a carbon-rich substrate) is suggested in literature as for AD processes optimization [120].

Figure 3.7: Brewery yeast [117]

3.2.5 Slaughterhouse waste

Slaughterhouse waste (fig. 3.8) is a biodegradable waste, that mainly consists of blood, manure, offal and paunch contents of animals. The slaughtering process involves different steps, such as animal killing, carcass removal, stomach and intestines cleaning [121]. The bloodstream is highly concentrated in BOD and COD; obviously, in this stream pollutants concentration is consistently higher than in washing water (produced from site washing) [122]. In literature, typical observed COD in slaughterhouse waste ranged from 18 to 43 g/L [123], even if it could reach levels as high as 100 g/L, depending on the waste composition and dilution [124]. However, for the purposes of this study, only the liquid organic waste from a local Friuli-Venezia Giulia slaughterhouse, mainly consisting of blood, was studied and analysed.

Similarly to what happens in dairies, slaughterhouse waste treatment and disposal is costly and difficult to apply locally, in particular for little facilities; in addition, due to the possible risk of biological contamination, many sanitary protocols should be observed in all process phases.

European Union (EU) legal framework for slaughterhouse byproducts management is mainly represented by European Regulation (EC) No. 1069/2009. Animal byproducts (ABP) consist of full bodies or parts of animals, as well as products of animal origin that are not intended for human consumption, either because they are improper for consumption, as a result of their nature, or due to a lack of commercial demand. More specifically, ABP are classified into three

Figure 3.8: Slaughterhouse liquid waste [125]

categories, namely Category 1, 2 and 3, which reflect the degree of risk, on the basis of a risk assessment procedure [126].

ABP are characterized by high organic carbon contents and are rich in lipids and proteins, thus making them an interesting feedstock for AD, due to the elevated potential for biogas production. On the other hand, the composition of ABP may sometimes slow down the hydrolysis step, and consequently the overall process rate.

In fact, fat hydrolysis results in the production of glycerol and long chain fatty acids, whose accumulation has been associated with possible methanogenic activity inhibition [127]. In addition, high fat contents may cause flotation and biomass washout. Furthermore, inhibitory effects, due to high ammonia concentration (result of protein degradation), may also manifest. Therefore, anaerobic treatment of slaughterhouse waste can be a good solution for a proper management of this waste, even if an efficient process control is required, due to the high protein and lipid concentration of the waste, to avoid generation of floating aggregates, that consequently increase effluent COD [128].

3.2.6 Sonication pre-treatment

Ultrasound (US) is a sound wave at a frequency range from 20 kHz to 10 MHz, and has a wide range of environmental applications [129]. A typical scheme

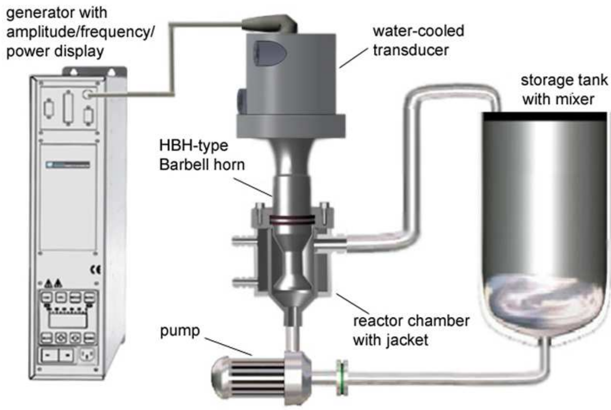

Figure 3.9: Example of industrial-scale US equipment [132]

of industrial-scale sonication equipment is reported in fig. 3.9. In WW sludge management, low frequency US pre-treatment, prior to sludge AD, is one of the most promising technniques, and it has been extensively investigated in recent years [130]. It has been demonstrated that US pre-treatment of both primary and secondary sludge enhances AD performances, by accelerating the hydrolysis step, due to an increase in bioavailable substrate concentration [131].

Beside sludge management, in the last decade, there has been a growing interest in US application to water and industrial WW treatment, to improve biodegradability and reduce the toxicity of different industrial WWs.

However, total mineralization of organic pollutants, by means of US irradiation, still remains a difficult task to achieve, and thus US application at industrial scale is impractical [134]. To overcome the limitation of low degradation efficiency, many efforts have been made on investigating various combined US systems (such as US+H_2O_2, O_3, electrochemical methods, Fenton reagent, photocatalysis), in order to reach the desired efficiency, reducing, at the same time, the required reaction time [135].

A new and upcoming approach is the use of US in food and dairy processing industries; in these cases, typically low frequency US is applied (20 kHz), to generate shear and turbulence. In the specific case of dairy industry, US pre-treatment can be applied, from a theoretical point of view, to increase methane production in AD processes; in addition, sonication can be used for process improvement in different operating phases (pre-treatment, ultrafiltration, spray drying and crystallization) in recovery of valuable products from CW, where membrane ultrafiltration is applied [136]. The first approach will be used in this chapter, and also in BMP tests (Chapter 4), while the second approach will be more deeply described in Chapter 6.

In fact, US was tested, in this work, on CW, with the aim of disaggregating solid matter and large macromolecules (abundantly present in this substrate), producing lower molecular weight compounds, more easily biodegradable. This could potentially enhance hydrolysis rate in anaerobic digestion, improving not only Biochemical Methane Potential (BMP) value, but also CH_4 production kinetics.

A laboratory-scale equipment was used, and separate characterization of sonicated and untreated CW was done, in order to underline meaningful differences in physicochemical properties. Moreover, BMP tests were executed on sonicated and non-sonicated whey (Chapter 4). Furthermore, given the undoubted importance of resource recovery, in particular from CW, the process of protein and lactose recovery from CW will be deeply analysed in Chapter 6.

3.3 Materials and methods

3.3.1 Inoculum and substrates

All the substrates were withdrawn from local facilities (Enemonzo and Ovaro cheese factories, fig. 3.10, Tolmezzo P&P factory, Sauris brewery, Amaro slaughterhouse), located in the Carnia territory (mountain area of Friuli-Venezia Giulia region) and were fully characterized, both using traditional and macromolecular parameters. First and second CW were separately collected and analysed, to underline the differences between these two matrices. Moreover, due to the limited number of data in scientific literature regarding detailed characterization of first and second whey, the analysis were repeated over a period of time, to obtain robust results.

Granular sludge was taken from UASB reactor, located in Tolmezzo WWTP, while anaerobic flocculent sludge, used for some BMP tests, in particular on brewery waste, was withdrawn from a full-scale anaerobic digester, located in Udine WWTP (200,000 PE).

OFMSW was collected from Udine University canteen, manually selected, to remove non-biodegradable materials, such as plastics or bones, and treated in the percolation bed without delay. In this work, the percolation bed was

Figure 3.10: Artelatte cheese factory (Ovaro) [137]

used only as a pre-treatment of the waste, to separate leachate from residual solid waste (as described by [138]); no inoculum was added to the leaching bed, differently from the work reported in [102] and [139], where a two-stage AD process was implemented.

The percolation bed was assembled as shown in fig. 3.11: the waste was dimensionally reduced and put over a porous tissue, which allowed water to stay in contact with the solid waste, and slowly percolate in the lower part of the bed; in addition, a layer of sand and gravel was put under the tissue, to allow a better separation of solid particles. The liquid fraction was extracted after the desired contact time, by means of a silicon tube and a peristaltic pump.

A defined protocol was adopted, in order to make the tests reproducible: 1.4 kg of selected OFMSW were put in the percolation bed, adding 1.5 L of tap water. After 24 hours, the leachate was extracted, and other 1.5 L of water were added. Then, the leachate was extracted after other 24 hours, and the 48-hour mixture of first and second day leachate was used for laboratory analysis and BMP tests. Globally, water-to-waste ratio was calculated to be 2.1:1 [140].

Three different OFMSW granulometries were tested in the leaching bed: untreated waste (i.e. without dimensional reduction), ground waste (particle diameter=12 mm) and pulp waste (particle diameter <0.5 mm).

Figure 3.11: Percolation bed for leachate extraction from OFMSW

3.3.2 Analytical methods

All the analysis were performed following Standard Methods for Examination of Water and Wastewater [141]; in particular, the following parameters were measured: tCOD, sCOD, TS, VS, pH, alkalinity, TKN, NH_3-N, $PO_4{}^{3-}$-P, $SO_4{}^{2-}$, VFA, macromolecules (carbohydrates and lipids) [140].

Carbohydrates were analysed using Dubois method [142], with glucose as standard. Lipids were measured by gravimetric analysis, after acetone hexane extraction. Protein concentration was calculated from TKN and NH_3-N concentration; nitrogen-to-protein conversion factor was approximated, for all substrates, to 6.25, except from CW, where a value of 6.38 was used, coherently with literature recommendations [143].

Volatile Fatty Acid (VFA) concentration was determined by gas-chromatography, with mass spectrometer (Agilent 6890 Plus/5973N) equipped with capillary column (Agilent HP-5MS).

Elemental analysis (C, H, N, S) was performed for dried samples (at 108 °C, overnight) on an elemental analyser (Flash EA1112, ThermoQuest/CE Elantech, Lakewood, NJ) (as previously done by [144]). It was conducted using automated combustion/reduction at 900 °C, followed by molecular sieve gas chromatography at 60 °C and thermal conductivity detection system.

Mean value will be reported for all physicochemical parameters; for each parameter, triplicate analysis were conducted, and standard deviation was <10%.

3.3.3 Ultrasound tests

Up200St (Dr. Hielscher) (fig. 3.12) was used for US tests; this device was characterized by maximum power of 250 W, working frequency of 26 kHz, oscillation amplitude of 20%. Before starting the tests, calibration was performed, both using thermal (measuring T increase in water at different powers) and chemical method (measuring absorbance of KI solution, at different US time); a good linearity was observed, in particular at low treatment power (in the range of 40-160 W).

Treatment time, in this first phase, was fixed at 200 s, corresponding to 50 kJ of energy transmitted to the substrate, and the device was used at the maximum power, to enhance disaggregation of larger molecules. The matrix to be treated was introduced in a specific beaker, in 250 mL aliquots, and was then sonicated, maintaining the sonotrode at the same depth for all tests, to obtain reproducible conditions.

Specific energy transmitted to the samples was calculated: starting from cheese whey density (1020 kg/m^3), the sonicated mass during each cycle was 255 g; taking into account mean TS content of whey (6.6%), that was obtained from physicochemical characterization results, the sonicated TS for each cycle were then estimated as 16.83 g TS. So, the specific energy transmitted to the sample was 2,971 kJ/kg TS, coherently with the range used in other literature studies [145].

3.4 Results and discussion

3.4.1 Physicochemical characterization

The results from physicochemical characterization of selected substrates (CW, condensate water, OFMSW leachate, brewery waste, slaughterhouse waste) were summarized in tab. 3.1. As for leachate, the liquid obtained from untreated ($OFMSW_u$), ground ($OFMSW_g$) and pulp ($OFMSW_p$) waste percolation was separately analysed. As for brewery waste, the results from the analysis on trub, yeast and end-of-fermentation beer were reported, given the fact that whirlpool residue gave similar results to trub.

Figure 3.12: Up200St (Dr. Hielscher) US equipment [146]

All the substrates had a greater COD value than condensate water, that represents the project stream for UASB reactor in Tolmezzo WWTP. In fact, it could be noticed that sCOD increased from 4.15 g/L, in condensate water, to a range of 15.1-37.4 g/L, in OFMSW leachate, 26.0-41-0 g/L, in brewery waste, 62.5-68.5 g/L, in CW, and 109.8 g/L, in slaughterhouse waste. tCOD was 105.0 g/L in first whey, while it was 81.8 g/L in second whey: it was inferred that first whey had a higher particulate fraction than second whey, leading to a greater tCOD. The further processing of first whey, in fact, probably allows some sedimentation of solid matter and COD. Instead, in OFMSW leachate tCOD was in the range of 17.9-40.0 g/L, indicating a low amount of particulate fraction, while this parameter was not detected in slaughterhouse waste and brewery waste, because of the extremely high solid content of this matrices.

pH was mainly acidic in all selected substrates, even if pH was lower in condensate water (3.5) than in leachate (4.6-5.2), CW (5.5-5.8), brewery waste (5.2-6.1) and slaughterhouse waste (7.2). Granular sludge showed a neutral pH (6.9).

Alkalinity was substantially absent in condensate water (<5 mg $CaCO_3$/L), low in leachate (443-538 mg $CaCO_3$/L) and brewery waste (298-905 mg $CaCO_3$/L), moderate in CW (1,153-1,297 mg $CaCO_3$/L) and very high in slaughterhouse waste (11,100 mg $CaCO_3$/L). It should be reminded that some alkalinity is needed in anaerobic processes, to prevent any pH drop.

As for nutrients, low NH_3-N concentration was found in all substrates, starting from condensate water (<1 mg N/L), passing to slaughterhouse waste (19.6 mg N/L), CW (3.19-44.1 mg N/L) and OFMSW leachate (24.8-41.6 mg N/L). The highest NH_3-N concentration was registered in brewery waste, in particular yeast (160 mg N/L). PO_4^{3-}, instead, were totally absent in condensate water (0.05 mg P/L), moderate in leachate (51-86 g P/L), high in slaughterhouse waste (173 mg P/L) and brewery waste (200-280 mg P/L), very abundant in CW (527-530 mg P/L).

TKN concentration, representative of the totality of N compounds, was very high in slaughterhouse waste (2,160 mg N/L), moderate in leachate (241-405 mg N/L) and first whey (332 mg N/L), low in condensate water (80 mg/L) and second whey (28 mg/L).

Sulphate concentration was negligible in slaughterhouse waste and first whey (<2 mg/L), while it was appreciable in condensate water (17.3 mg/L), leachate (16.6-20.2 mg/L) and second whey (55.5 mg/L). In brewery waste, a significant difference between sulphate concentration could be highlighted: while trub did not contain significant sulphate (15.1 mg/L), high SO_4^{2-} was measured in yeast (65.5 mg/L) and, mostly, in end-of-fermentation beer (180 mg/L).

TS and VS concentration was again highly variable: condensate water had the lowest TS concentration (0.018% w/w), followed by leachate (1.41-3.23% w/w), CW (6.63-7.44% w/w) and slaughterhouse waste (15.11% w/w). Again, as could be imagined, significant variations in solid concentration were registered in brewery waste: end-of-fermentation beer had a low TS content of 3.77% w/w, while trub (15.99% w/w) and spent yeast (18.29% w/w) had higher TS. VS/TS ratio, that represents solid matter biodegradability, was fairly high for all the analysed substrates: the minimum percentage was found in leachate (66.9-79.5%) and brewery yeast (76.35%), while the maximum one was encountered in slaughterhouse waste (94.6%) and brewery trub (97.15%).

VFA concentration, indicative of an on-going acidification, was low in condensate water (38 mg/L) and CW (1-41 mg/L), while it was significant in leachate (56-158 mg/L) and, mainly, in slaughterhouse waste (820 mg/L).

Elemental analysis (fig. 3.13), executed on TS, highlighted the following aspects:

- UASB sludge (9.7%) and condensate water (12.1%) were predictably rich in S;
- Anaerobic sludge from Udine WWTP had very low C content (26.4%), if

Table 3.1: Results from physicochemical characterization of selected substrates

Parameter	Sludge	Conden	1^{st} CW	2^{nd} CW	$OFMSW_u$	$OFMSW_g$	$OFMSW_p$	Trub	Yeast	Beer	Slaught
tCOD (g/L)	n.d.	4.15	105.0	81.8	17.9	40.0	26.5	n.d.	n.d.	n.d.	n.d.
sCOD (g/L)	1.73	4.15	68.6	62.5	15.1	37.4	22.2	41.5		26.0	109.8
Alka (mg $CaCO_3$/L)	1873	<5	1297	1153	490	443	538	298	905	322	11100
NH_3-N (mg N/L)	179	<1	44.1	3.19	24.8	36.5	41.6	78.3	160	65	19.6
TKN (mg N/L)	216	80	332	28	241	405	291	n.d.	n.d.	n.d.	2160
PO_4^{3-}-P (mg P/L)	30.8	0.05	530	527	67	86	51	280	n.d.	200	173
SO_4^{2-} (mg/L)	<2	17.3	<2	55.5	16.6	20.2	<2	15.1	65.5	180	<2
pH	6.9	3.5	5.5	5.8	5.2	4.6	4.9	5.8	6.1	5.2	7.2
TS (% w/w)	4.18	0.018	7.44	6.63	1.41	3.23	2.22	15.99	18.29	3.77	15.11
VS (% w/w)	3.79	n.d.	6.73	5.64	0.94	2.57	1.53	15.53	13.96	3.56	14.29
VS/TS (%)	90.59	n.d.	90.37	85.18	66.99	79.52	68.91	97.15	76.35	94.47	94.57
VFA (mg/L)	110	38	41	1	109	56	158	n.d.	n.d.	n.d.	820

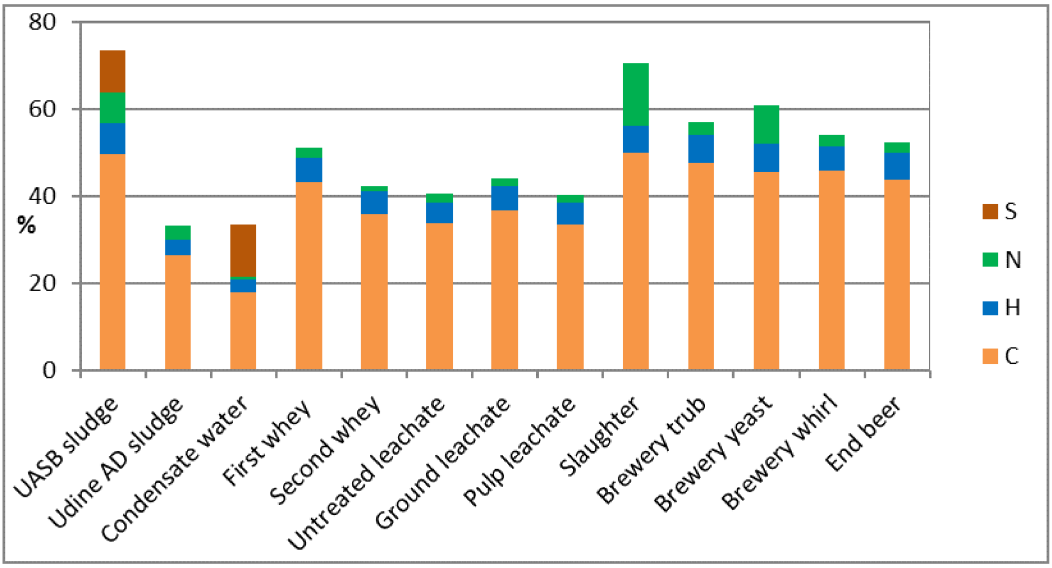

Figure 3.13: Results from elemental analysis on selected substrates

compared to granular sludge, and contained no S;

- Slaughterhouse waste was characterized by high N content (14.6%);
- First whey had a higher C content than second whey (43.1% vs 35.8%);
- OFMSW leachate, despite of the different waste particle dimensions, had very similar composition (C=33.6-36.7%; H=4.9-5.7%; N=1.6-1.9%);
- Brewery waste had high C content (43.7-47.5%) and a significant N percentage (8.7%) was found in spent yeast.

In tab. 3.2, C/N ratio was reported, as resulting from elemental analysis. It could be highlighted that anaerobic sludge had a similar C/N ratio (7.0-8.5), despite of the different origin and characteristics, while a very low C/N ratio (3.4) appeared in slaughterhouse waste. Spent yeast had a low C/N ratio of 5.2, while higher C/N ratio was instead registered both in CW (18.3-35.8), leachate (17.7-22.9), brewery trub, whirlpool, end-of-fermentation beer (16.3-17.0) and, mostly, condensate water (35.5).

Table 3.2: C/N ratio from selected inocula and substrates

Matrix	C/N ratio
UASB sludge	7.0
Udine sludge	8.5
Condensate water	35.5
First whey	18.3
Second whey	35.8
Untreated leachate	17.7
Ground leachate	22.9
Pulp leachate	19.1
Slaughter	3.4
Trub	16.4
Yeast	5.2
Whirl	17.0
Beer	16.4

A balanced C/N ratio, in the range of 20-30, is expected to favour stabilization of conditions inside anaerobic digesters; low C/N can lead to excessive NH_3 concentrations, impeding microbial growth, while, on the other hand, excessive C/N stimulates VFA build-up [147].

Macromolecular analysis results (fig. 3.14), in addition, revealed that:

- UASB sludge was rich in lipids (4.55 g/L);
- Condensate water, as expected, was generally poor in all macromolecules;
- The main difference between first and second whey was in lipid concentration, that was noticeably higher in first whey (33.0 g/L versus 2.4 g/L);
- Leachate was mainly composed of carbohydrates (6.1-15.2 g/L);
- Slaughterhouse waste was rich in proteins (13.5 g/L) and mostly in lipids (110.3 g/L).

It should be reminded that carbohydrates are believed to be readily biodegradable in anaerobic environment, while lipids, despite being able to produce higher

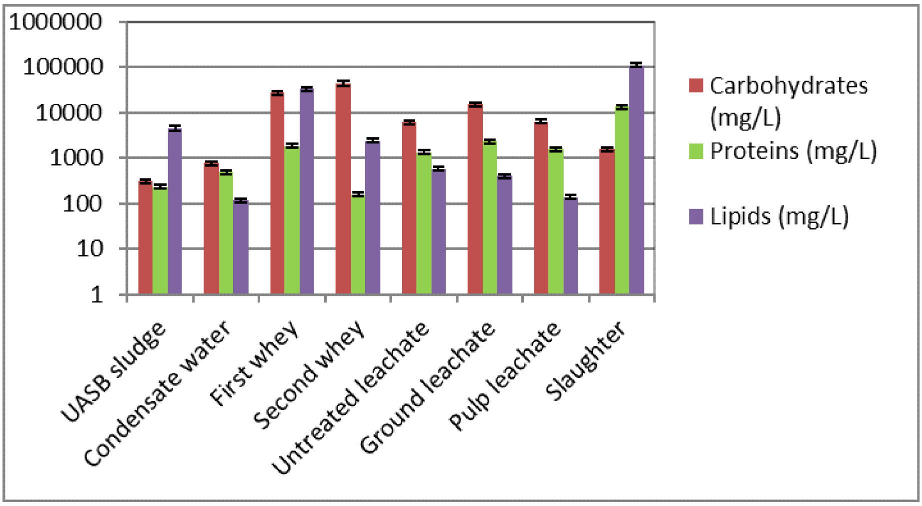

Figure 3.14: Results from macromolecular analysis on selected substrates

methane yields, are slowly degraded, and often accumulate in the reactor, leading to operational problems, such as sludge floatation and foam formation [39]. Proteins are degraded to amino acids, that subsequently form NH_3; as a result, an excessive protein concentration can cause inhibitory effects [64].

So, it can be inferred that macromolecular composition of slaughterhouse waste is not particularly favourable for AD, because its anaerobic treatment is expected to favour a build-up of high NH_3 concentrations, and a low lipid abatement would probably be obtained, with foam formation. On the other hand, leachate, having high carbohydrate concentration, but also CW (in particular second whey) showed a good macromolecular composition, and they are expected to be anaerobically treated in an efficient manner, with stable operating conditions.

3.4.2 Discussion

Cheese whey

The work by Escalante et al. [86] underlined the extreme variability of CW properties, due to the different origin of the milk used for cheesemaking process, as well as to the peculiar operations performed in each dairy. They reported VS of 40-65 g/kg (coherent with the range of 56-67 g/kg measured in this study) and COD of 65-140 g/L (the analysed whey showed an intermediate range of 81.8-105.0 g/L). In addition, they reported two ranges in VFA concentration, one between 8 and 10 g/L, and the other between 2 and 5 g/L, while other literature findings reported a lower range of 0.5-5.45 g/L [148]. However, measured VFA concentration in the collected whey samples was consistently lower than typical literature range. This high VFA concentration from literature evidences underlined that CW is a substrate capable of producing high methane yields, but with a notorious lack of alkalinity, that can lead to acidification during AD processes [149].

As for pH, in [86] an acidic range of 3.0-6.5 was reported, well fitting with the results of the current analysis (5.5-5.8), that highlighted a mild acidity in whey. Generally speaking, a lower pH is typically associated with a higher VFA concentration. Erguder et al. [150] reported an acidic pH in whey (3.44-3.92), together with a lower COD concentration (55.3-74.5 g/L) than that of the present work; also, the reported PO_4^{3-} concentration from their work (124 mg/L) was lower than the actual results.

Blonskaja and Vaalu [151], instead, studied different substrates, including CW, and reported a COD range of 60.3-66.7 g/L, lower than that of the present study, while BOD was in the range of 35.5-46.0 g/L. Dry matter and pH, instead, were coherent with the results of the actual characterization, having respectively the ranges of 5.7-7.1 g/L and 3.8-6.3.

It could be concluded that a great variability in reported physicochemical properties of CW emerged from literature studies, but it can be easily explained, because this substrate is strongly heterogeneous, and its composition depends both on the characteristics of raw milk (type of animal and feed), as well as processing conditions, that are adjusted case by case in each dairy plant, to obtain the desired products. In addition, these variations can be further exacerbated, if little dairies are considered, such as the ones present in Friuli-Venezia Giulia mountain area.

Condensate water

Due to the particular operations performed in Tolmezzo P&P factory, that include lignin-sulphonate concentration, from which condensate water is originated, it is difficult to find meaningful comparisons with other literature studies. However, in the work by Meyer and Edwards, several pulp and paper mill streams were presented and characterized; in particular, they reported, for kraft combined condensates, a COD range of 0.7-4.0 g/L, coherent with the analysed condensate water. Moreover, a high sulphide concentration (210 mg/L) was highlighted, that could be probably encountered also in Tolmezzo stream, before the stripping tower. Furthermore, they reported methanol as the main organic compound in the stream, having concentration of 1.3 g/L, while TSS concentration was very low (12 mg/L), well fitting with the results from the stream considered in this research (in Chapter 2, a SS concentration of 20 mg/L was reported for condensate water).

OFMSW leachate

Campuzano et al. [138] used ground waste for percolation, and tested different waste-to-water ratios (1:1, 1:2, 1:3), coherent with the ratio of 1:2.1, that was used in the laboratory tests. The reported results of their study (fig. 3.15) showed that, after 30 min of contact between water and organic waste, no further solubilisation occurred; in addition, the water extracts exhibited a high VS/TS ratio (83.4-88.9%), greater than that reported in the present work. This could be explained with a worse retention of inorganic sand particles in the actual leach bed, so some particles could be dragged with the leachate. However, it must be noticed that there are many variables affecting leachate characteristics, starting from OFMSW composition (highly dependent from producing facilities and seasonality) and particle dimensions, as well as percolation bed configuration, waste-to-water ratio and contact time; so, it is really difficult to standardize this substrate.

Nayono et al. [153] characterized press water, obtained after OFMSW mechanical pressing, and they obtained a very high COD (tCOD=213.4 g/L, sCOD=100.1 g/L), significantly higher than that of the studied leachate; moreover, also TS (168.4 g/L) and VS (117.7 g/L) of press water were higher than that of the obtained leachate. In addition, significant TKN (4.1 g/L), ashes (50.7 g/L) and acetic acid (8.56 g/L) concentrations were reported. The accelerated acidification process was underlined, given the high concentration of total VFA (9.51 g/L), where acetic acid was the predominant compound [153]. Thus, it could be seen that a higher load is obtained, when using mechanical separation, instead of percolation; this can be a good point also for increasing obtainable methane yields in AD processes.

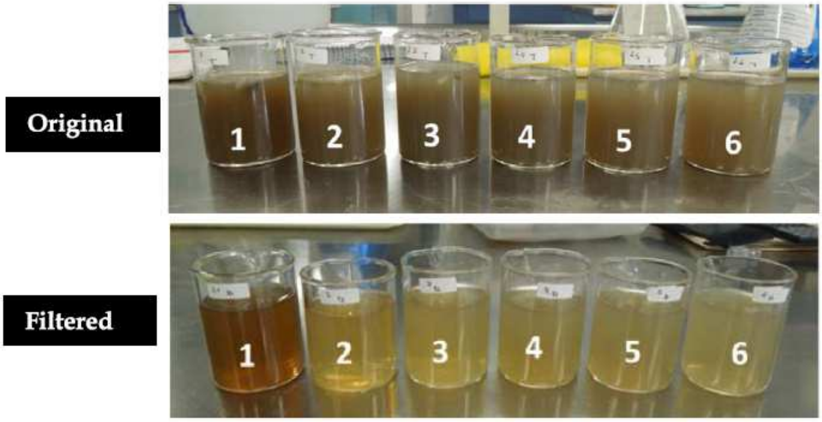

Figure 3.15: OFMSW leachate, derived from 6 successive extractions [152]

Brewery waste

Panijicko et al. [120] reported a higher TS content (21.1-26-3%) in BSG, if compared to the actual results; this could be explained with a better separation of residual liquid fraction from trub. VS/TS ratio, instead, was 96.1%, that was similar to that obtained in the actual study. Moreover, they claimed TN concentration in the range of 11-13 g/kg wet weight; the prevailing compounds in BSG were showed to be hemicellulose (24.7% of TS), cellulose (23.7% of TS), lignin (24.6% of TS) and protein (21.4% of TS). Bougrier et al. [154], instead, reported again a high TS concentration in BSG (24.4%), similar to that reported in [120].

As for spent yeast, in [109] a dry matter content of around 10% was reported, lower than that obtained in the present study.

In [120], brewery wastewater was characterized, having a pH of 6.8-7.1 (significantly higher than measured pH in this work) and a very low TS concentration (90-280 mg/L). Moreover, as for brewery wastewater, in [109] a nitrogen concentration of 30-100 mg N/L was reported (measured NH_3 in end-of-fermentation beer fell in this range), while, as for P, a similar range of 30-100 mg P/L was claimed (actually, measured phosphate concentration in end-of-fermentation beer was significantly higher).

Slaughterhouse waste

Again, a high variability was reported in literature for slaughterhouse waste, because of its extreme heterogeneity; slaughterhouse waste could contain different animal residues, and also the operating conditions in the slaughterhouses are not standardized at all. Ahmad et al. [124] reported in their work a COD concentration ranging from 27.1 to 101.0 g/L, lower than that measured in this study; Moukazis et al. [126] studied 4 different ABPs from slaughterhouses: the stomach contents characterization showed similar TS (13.5%) and VS (11.7%) content to that of the analysed waste, even if the studied waste was mainly composed of blood, so its origin was not comparable at all. Moreover, as for elemental analysis, their matrix had a similar C (50.1%) and H (5.8%) percentage to the actual waste, while they reported lower N (3.6%) and higher S (4.4%). In this study no S was detected in the slaughterhouse waste.

3.4.3 US pre-treatment

As previously stated, characterization of some meaningful parameters was done for untreated and sonicated samples, in order to understand the possible influence of this pre-treatment on AD processes. In particular, US was tested on a single first whey sample and two distinct second whey samples, collected during different times of the year, to consider the variability in whey characteristics. The results, reported in tab. 3.3, highlighted that in first whey an interesting reduction in lipids (-95%) and carbohydrates (-17%) was observed after sonication, together with an high increase in TKN (indicating protein degradation). No VFA increase was observed.

As for sonication results on second whey, significant differences in macromolecular composition were observed in the analysed samples; again, a moderate carbohydrates (4-11%) degradation was observed, while lipid degradation, similarly to first whey, was more consistent (36-86%). A significant increase in TKN concentration was noticed, and VFA concentration increased, at the same time: in one sample, there was an increase as high as 183%, while in the successive sample the increase was moderate (11%).

From this preliminary tests, it could be stated that US is expected to reduce, in particular, lipid and protein concentration, while its effect on carbohydrates is negligible. However, a more in depth analysis is required, to better understand the effective modification induced from US pre-treatment on physicochemical properties of CW. In fact, it should be underlined that, given the high expense for these macromolecular tests, only a limited number of analysis could be done.

Table 3.3: Characterization of untreated and sonicated cheese whey samples

Matrix	VFA (mg/L)	Lipid (g/L)	Carb.(g/L)	TKN(mg/L)
First whey	1	2.388	43.8	28
US 1^{st} whey	1	0.110	36.2	493
Second whey (I)	41	32.955	27.5	332
US 2^{nd} whey (I)	116	4.680	24.4	997
Second whey 2(II)	90	6.720	41.7	120
US 2^{nd} whey (II)	100	4.020	40.0	1130

3.5 Conclusions

Some useful indications were obtained from literature study and laboratory physicochemical analysis of the selected substrates. In particular, it was noticed that:

- Condensate water is a nutrient deficient substrate, highly acidic, and without significant solid matter; it is generally poor in macromolecules and has an optimum COD concentration for UASB process. Elemental analysis underlined the high S concentration in the scarce solid matter content of this matrix, together with the high C/N ratio. Moreover, its high T is already optimal for mesophilic AD processes, without any heat requirement;

- CW has a COD concentration (80-100 g/L) greater than typical values used in high-velocity processes, and so a useful dilution should be planned in continuous UASB tests. A high heterogeneity in physicochemical properties was underlined, also coherently with literature evidences. A mild acidic pH was observed, together with a high phosphate and low ammonia concentrations. First whey was generally richer in lipids, if compared to second whey; US pre-treatment had an interesting effect in reducing lipid and protein concentration, while its effect on carbohydrates was negligible;

- OFMSW leachate had a high COD value, however highly dependent on leaching bed operating conditions, such as waste origin, characteristics, granulometry, as well as percolation bed configuration (water-to-waste ratio, contact time). Its low solid matter content and its significant carbohydrate concentration indicated a general good biodegradability, useful for AD processes;

- Brewery waste (BSG, yeast, whirlpool, end-of-fermentation beer) was characterized by high COD (26-134 g/L), variable TS content (from 3.77% in beer, up to 18.29% in yeast), substantial acidity and low alkalinity. The high biodegradability of brewery residues, as highlighted also from literature evidences, can be fruitfully exploited in AD processes;

- Slaughterhouse waste had an extreme solid matter concentration (15.11% TS), coupled with very high organic matter content (sCOD up to 109.8 g/L) and significant protein (13.5 g/L) and lipid (110.3 g/L) concentrations, that are expected to create operational problems in continuous systems, such as NH_3 accumulation and foam formation. Moreover, it should not be forgotten that sanitary protocols should be taken into account, in its proper treatment and disposal.

In Chapter 4, given the obtained physicochemical properties of the analysed substrates, the results from BMP tests will be presented, in order to establish potential methane recovery from each matrix. The results from this chapter will be particularly useful in selecting correct I/S ratios and proper inoculum for each test.

Chapter 4

BMP tests

In this work, different inocula were used for BMP tests, depending on the substrate characteristics. In fact, a first series of tests was executed on CW, condensate water, OFMSW leachate and slaughterhouse waste using granular UASB sludge, withdrawn from Tolmezzo WWTP, as inoculum; successively, a further series of tests was done on the same substrates, with a different granular sludge, taken from an Internal Circulation (IC) reactor, that is merely an evolution of classical UASB, located in Castelfranco Veneto (Tv). Then a comparison was made, in order to evaluate the unavoidable activity loss of a sludge, such as that of Tolmezzo plant, that has been inactive for nearly 10 years. Further tests were planned on CW, where a broader spectrum of I/S ratios was experimented, to evaluate the best operating conditions, using the highly active granular IC sludge.

US pre-treatment was evaluated on CW, as a useful tool to increase methane yields in AD process: to this purpose, different US conditions, in terms of applied power and time, were tested, to study an eventual correlation between applied US energy and increase in BMP value. An energetic analysis was then made on the obtained results, to convert BMP increase in potential methane yield.

Furthermore, BMP tests were made on brewery waste (spent grain, yeast, end of fermentation beer and whirlpool residue) using flocculent AD sludge, from full-scale traditional batch digester, located in Udine WWTP (200,000 PE), that actually stabilizes excess sludge. This sludge was used because of the high solids content of brewery waste, that, excluding end-of-fermentation beer, cannot be efficiently treated using granular UASB processes. Granular activated carbon (GAC) and biochar were added to selected BMP tests (in particular as for yeast and whirlpool residue), in order to evaluate a possible increase in methane production and propose a synergistic effect with thermal processes.

Finally, a kinetic analysis was performed on BMP test results, applying first-order kinetic model and modified Gompertz equation, and some meaningful parameters for AD modelling were inferred, such as estimation of hydrolysis constant and lag phase duration.

4.1 Introduction

CH_4 production potential from a generic substrate, suitable for undergoing an AD process, can be investigated by means of a simple Biochemical Methane Potential (BMP) test (schematically represented in fig. 4.1), that allows the determination of biodegradability and the associated methane production potential during AD of a given substrate [155]. Several studies were carried out over the last years, demonstrating an increasing interest in methods for an accurate measurement of the BMP of different substrates.

BMP assays provide an array of information on the substrate, including how fast and how much of the material can be degraded under optimal conditions, as well as its potential methane yield. Primary outputs of BMP assays are cumulative methane production curves (fig. 4.2), that are generally plotted against time. The tests duration depends on substrates biodegradability, and is generally in the range of 20-25 days.

The patterns that these curves follow are far from trivial, and have meaningful implications on substrate degradation [156]. With reference to fig. 4.2, reverse L-shape pattern is typically observed when digesting a readily-biodegradable substrate, where the organic matter is quickly converted to biogas; viceversa, an elongated S-shape trend appears when slowly degradable matrices are tested. Finally, a stepped curve can arise when the substrate is characterized by a fraction of readily available material, and another fraction of slowly degradable compounds.

The kinetics of the different stages of AD process (hydrolysis, acidogenesis, acetogenesis and methanogenesis), and ultimately the shape of methane production curves, are primarily controlled by the biodegradability characteristics of the substrate, the production of inhibitory intermediate fermenters and the performance of methanogenic bacterial populations [172]. The analysis of these curves can be significantly deepened using mathematic modelling of methane production kinetics, allowing further insight into substrate behaviour during AD process [156].

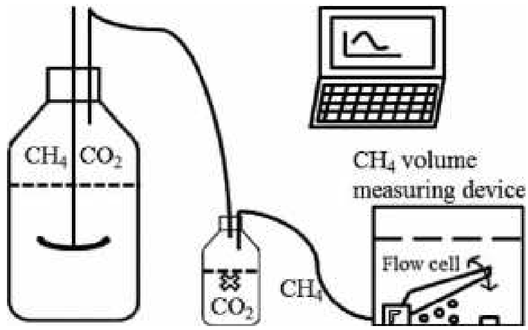

Figure 4.1: Schematic representation of BMP tests [157]

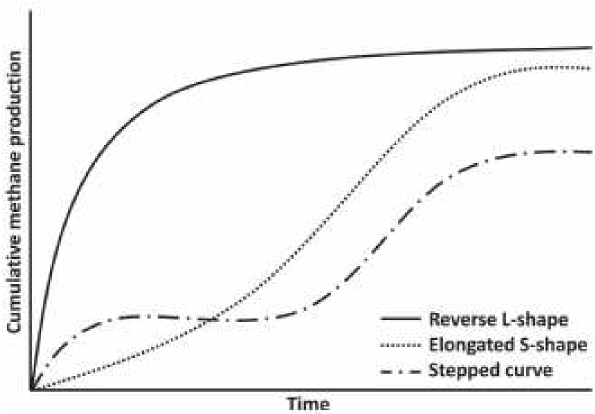

Figure 4.2: Examples of cumulative CH_4 production curves [156]

A raw estimation of methane production, based on substrate chemical composition, is possible but not very reliable, and so BMP measurement is generally preferred in scientific literature, even if standardization between literature data is complex, but necessary, to obtain robust, reproducible and universal results [158]. Guidelines for BMP determination in batch assays have been proposed by Angelidaki et al. [159], regarding substrate characterization, inoculum and activity, as well as experimental procedure and data collection, interpretation and reporting.

Raposo et al. [160] reviewed the factors affecting the performance of anaerobic batch assays, and indicated that, although experimental conditions are synchronized, a certain degree of variability in the results always remains, due to the biological nature of the systems. This biological difference can be attributed to inoculum origin, as it comes with a different microbial population, leading to differences in initial activity and adaptation to the substrate [161].

BMP tests are sensitive to operating conditions (temperature, pH, agitation intensity, inoculum to substrate ratio, i.e. I/S), as well as to substrates characteristics, such as particles size. I/S ratio, that indicates the relative ratio between inoculum and substrates amounts, is generally expressed on VS or COD basis, and is typically in the range of 1-3 (when calculated on VS), that is a value sufficiently high to reduce the lag phase (i.e., the time requested by the biomass to adapt to the substrate), but, on the other hand, also sufficiently low not to increase endogenous biogas production, that can false the results [162]. However, sometimes higher I/S ratios should be adopted, in particular when testing complex substrates, having high lignin or solid matter concentration.

BMP equipment, schematically represented in fig. 4.1, is a useful tool in order to simulate AD processes, even if continuous reactors, such as UASB, obviously cannot be well represented by a batch test. BMP tests can be a starting point, to study the interaction biomass-substrate, calculate methane potential from a given matrix and prevent possible operating problems, that can arise in continuous tests. BMP essays can give an indication of final methane yields and are also useful to study the kinetics of CH_4 production in the first digestion days, where the promptly biodegradable substances are expected to degrade [140]. Moreover, operating in standardised laboratory conditions can allow to establish a hierarchy of each substrate biodegradability in anaerobic environment, when multiple matrices are investigated.

Some preliminary considerations on UASB granular sludge characteristics and BMP tests can be made to conclude this paragraph, according to literature evidences. In the work by De Vrieze et al. [158], four different substrates were anaerobically digested with four different inocula, including a brewery wastewater

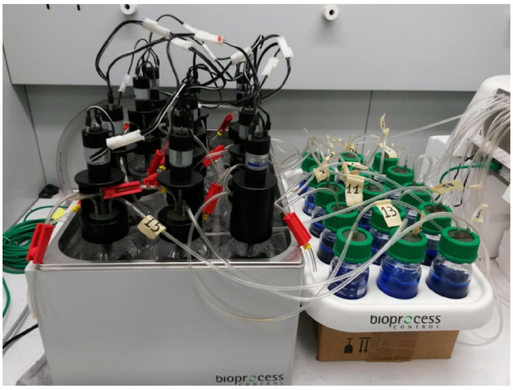

Figure 4.3: AMPTS equipment (Bioprocess Control)

inoculum, obtained from a UASB reactor. This inoculum produced the highest methane yield for three out of four tested substrates. Moreover, if compared to the other inocula, granular sludge showed a lower total bacteria abundance (by a factor of 10) and a higher total methanogens abundance (by a factor of 10), indicating high methanogenic activity.

4.2 Materials and methods

BMP tests were conducted using Automatic Methane Production Test System (AMPTS, Bioprocess) equipment. AMPTS (fig. 4.3) consists of 15 individual reactors of 650 mL volume, each equipped with a stirrer (connected to a motor controller); all the reactors are put in a thermostatic bath, maintained at the desired mesophilic temperature. The produced biogas is sent to an acid-fixation unit, that is a basic solution of soda, provided with a pH-indicator (tymolphtalein), that indicates solution saturation. Finally, the residual biogas (essentially pure methane) arrives to methane registration unit, that is formed by 15 injection mould flow cells, inserted in a further water bath, containing metal pieces, that open up and register every 10 mL volume of CH_4 [140].

I/S ratio was set, for the first series of substrates (CW, OFMSW leachate, slaughterhouse waste), at 2, and it was calculated on VS basis. In the case of condensate water, where a negligible solid matter content was observed, some preliminary evaluations were done, to establish an appropriate I/S ratio, and a value of 0.52 was chosen, calculated on sCOD. However, due to the fact that CW produced acidification in the first digestion tests, executed at I/S=2, successive tests were planned, using a variable I/S ratio (3, 5, 8), in order to get more reliable information about the obtainable methane yields from this substrate.

In addition, the effect of sonication pre-treatment on CH_4 yield was investigated, as well; sonication was performed, in a first run of tests, using the same US conditions described in Chapter 3 (maximum power and treatment time of 200 s), and a safe I/S ratio of 8. A variable range of US power (40, 80 W) and treatment time (5, 10 min) were used in a second phase, using an optimum I/S ratio of 6, in order to evaluate an eventual correlation between applied US energy and increase in BMP yield. The surplus in BMP yield was then converted in theoretical methane potential, assuming calorific value of methane as 10.30 kWh/Sm^3 and a conversion factor, from Nm^3 to Sm^3, of 1.056.

Finally, as for the tests executed on brewery residues, following some preliminary tests, that were useful to identify the best AD conditions for each substrate, an I/S ratio of 3 (calculated on VS) was adopted as for BSG (trub), while whirlpool residue, end-of-fermentation beer and spent yeast were tested using a higher I/S ratio of 6 (again calculated on VS).

Thermostatic bath temperature was set at 35 °C for all tests, and a discontinuous (30 seconds on-30 seconds off) mixing regime was set up. Before starting the tests, each reactor was flushed with nitrogen for 30 seconds, to establish full anaerobic conditions [163].

BMP tests were stopped when no methane production was observed for more than 24 hours. All the tests were done in triplicate, with a blank control. Final BMP value was calculated by subtracting methane production of the sludge alone, from methane production of the sample, and correcting methane production of the sludge with the actual amount of biomass in the sample bottles [140]. In fact, generally speaking, blank samples, even if are characterized by low biogas yield, play an important role, to assess the net gas production from a substrate [163].

All BMP tests were performed in triplicate; no pH correction or nutrient addition was performed, in order to analyse biomass adaptation to the substrates [140]. As previously mentioned, a large variety of BMP tests were executed, using 3 different inocula, namely Tolmezzo UASB sludge, Castelfranco P&P IC sludge and Udine classical AD sludge.

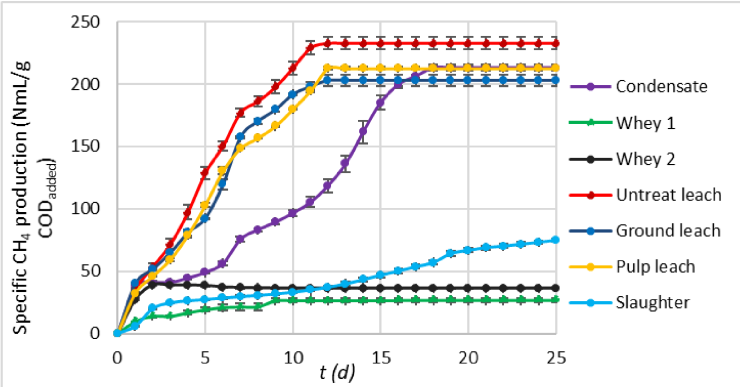

Figure 4.4: Results from BMP tests of selected substrates (Tolmezzo UASB sludge used as inoculum)

4.3 Results and discussion

4.3.1 BMP tests (Tolmezzo granular sludge as inoculum)

The results from the first series of tests, where granular sludge from Tolmezzo UASB reactor was used as inoculum, were summarized in fig. 4.4. As described in Chapter 3, this sludge was inactive since 2007, so it could be expected that its activity would be significantly reduced, if compared to a high-activity sludge.

BMP values obtained from selected substrates, using Tolmezzo UASB sludge as inoculum, were generally low, and this was obviously linked to the low activity of the granular biomass. As can be seen from fig. 4.4, final BMP value for condensate water was 213.1 L CH_4/kg COD_{added}, similar to that of OFMSW leachate (in the range of 212.7-232.8 L CH_4/kg COD_{added}), even if the kinetics of condensate water digestion was slower; this was probably due to the fact that leachate, being rich in carbohydrates, was more easily biodegradable than condensate water. In addition, BMP value from untreated, ground and pulp waste was very similar, indicating that particle dimensions did not significantly influence methane production; this could be expected also from the results of physicochemical characterization, that highlighted similar characteristics of the three leachates. Finally, as for slaughterhouse waste, methane production was very slow, and only after 15 days an appreciable raise in biogas yield was observed; final BMP was only 74.8 L CH_4/kg COD_{added}.

CW, finally, gave a scarce CH_4 production; a further set of analysis was executed immediately after the end of the tests, in order to understand what happened in the reactors, and it was highlighted that pH decreased to values as low as 4.0, so an intense acidification occurred, that inhibited methanogenic bacteria and stopped methane production. This was probably due to the extreme COD concentration of this matrix, coupled to an insufficient alkalinity: organic acids generation (acidogenesis) was much faster than their consumption (methanogenesis). A successive series of BMP tests was indeed planned, using different I/S ratios and the more active IC biomass (taken from Castelfranco plant) as inoculum; the results will be shown in paragraph 4.3.3.

4.3.2 BMP tests (Castelfranco granular biomass as inoculum)

BMP tests were repeated, at the same operating conditions (I/S ratio, substrate, T, mixing rate) using a highly active biomass, taken from a full-scale P&P factory, located in Castelfranco Veneto (fig. 4.5). For each substrate, the comparison between CH_4 yield with the 2 inocula was reported in fig. 4.6, 4.7, 4.8 and 4.9.

As for CW, again a low methane yield was observed, even lower than that obtained using Tolmezzo sludge; also in this case, a strong acidification happened. It should be concluded that, despite biomass origin and activity, a higher biomass amount is required in BMP tests, to provide sufficient buffering capacity to avoid methanogens inhibition, when digesting CW. Indeed, a higher spectrum of I/S ratios was used in further tests, to obtain reliable data on the effective CH_4 yield from this substrate. The influence of I/S ratio on cheese whey BMP value will be deeply analysed in the paragraph 4.3.3, where also the first results from sonication pre-treatment influence on BMP value will be presented.

As for condensate water, BMP value, and also methane production rate, was significantly higher, in the case of Castelfranco sludge, if compared to the previous tests: this could be explained with the high activity of the current inoculum, and, moreover, by considering the fact that condensate water was very similar to P&P WW effectively treated in Castelfranco plant, so biomass adaptation, in this case, was not needed. Condensate water, in fact, was the substrate which highlighted the highest difference in the two inocula performance: BMP curves, reported in fig. 4.7, showed that condensate water, tested with IC sludge, produced more than three times the CH_4 obtained with Tolmezzo UASB sludge (687 NmL CH_4/g COD_{added}, versus 213 NmL CH_4/g COD_{added}); in addition, a much faster kinetics was observed. Anyway, CH_4 production,

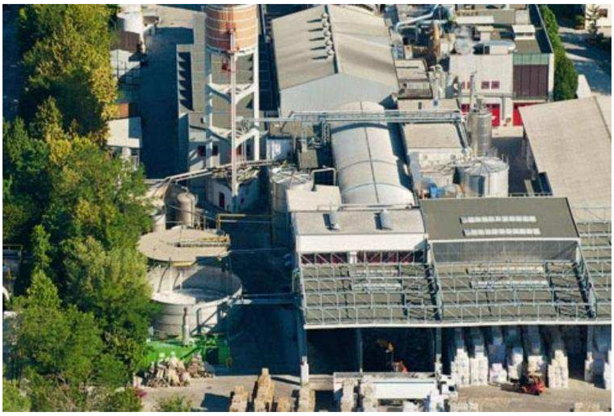

Figure 4.5: Castelfranco Veneto P&P factory [164]

despite the different biomass characteristics, similarly stopped after 17-18 days of tests.

As for OFMSW leachate, a similar trend from the two sets of tests was observed in methane production, as highlighted in fig. 4.8, even if a higher CH_4 production was obtained in the first 2 days, using the more active biomass, while in the successive days the low activity biomass outperformed the other one. However, final BMP value from leachate digested using the two inocula was very similar (216.5 versus 202.8 NmL CH_4/g COD_{added}). This behaviour could be explained with the substantial biodegradability of the substrate: in this case, CH_4 production was not greatly influenced from inoculum characteristics.

Finally, slaughterhouse waste tests (fig. 4.9) underlined again a higher methane production using Castelfranco biomass; in particular biogas generation was significantly higher in the first days of digestion, indicating a faster hydrolysis of complex organic material. Final BMP reached 162.8 NmL CH_4/g COD_{added}, using IC biomass as inoculum, instead of 74.8 NmL CH_4/g COD_{added}, that were obtained using UASB inoculum. However, slaughterhouse waste, among the tested substrates, was the one which produced the lowest CH_4 yield; consequently, it was chosen not to proceed with further tests on this matrix, given also the long times needed to obtain some meaningful indications from continuous tests, such as the ones presented in Chapter 5. Moreover, given the high solid content of this matrix, different inoculum should be used, to obtain higher methane

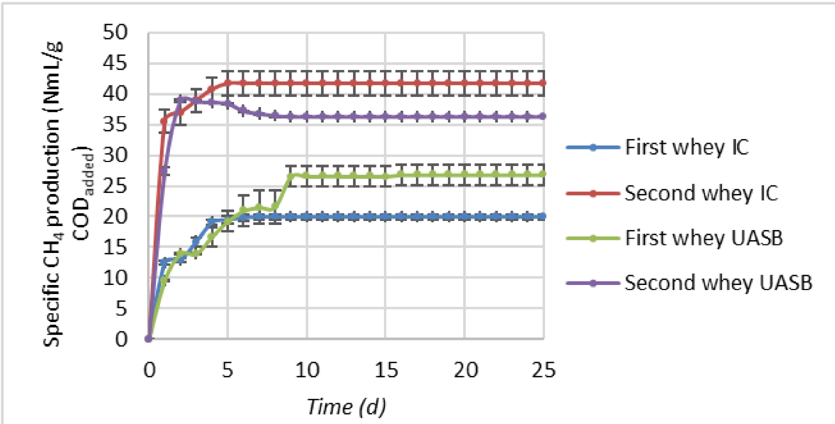

Figure 4.6: Comparison between BMP test results on cheese whey, using Tolmezzo (UASB) and Castelfranco (IC) biomasses as inocula

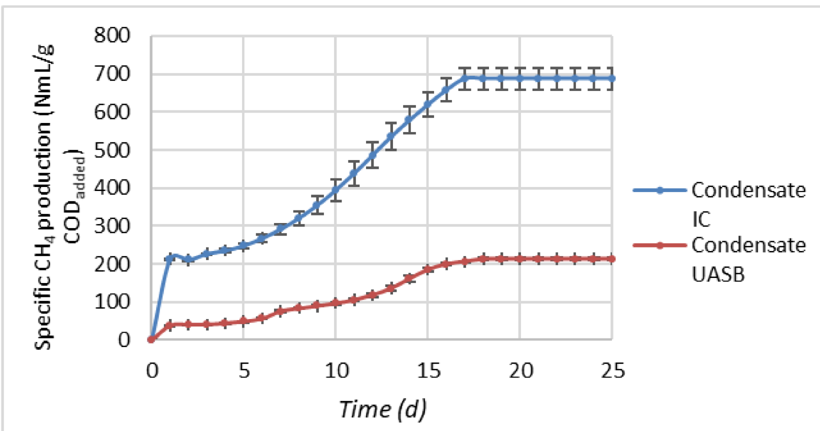

Figure 4.7: Comparison between BMP test results on condensate water, using Tolmezzo (UASB) and Castelfranco (IC) biomasses as inocula

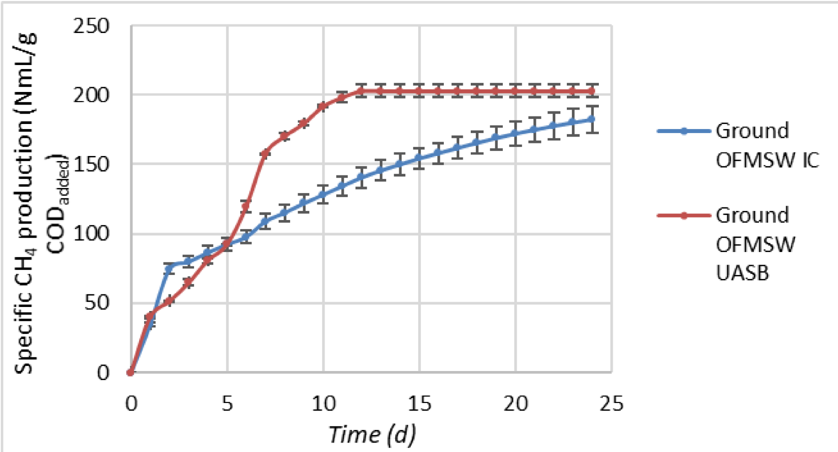

Figure 4.8: Comparison between BMP test results on OFMSW leachate, using Tolmezzo (UASB) and Castelfranco (IC) biomasses as inocula

yields.

4.3.3 BMP tests on CW using different I/S ratios

Because of the sudden acidification, that was observed on CW using an I/S ratio of 2, a successive series of tests was planned on this substrate, using a broader spectrum of I/S ratios (3, 5, 8); moreover, as previously described, sonication was tested on whey, at I/S=8, in order to verify if there could be an effective increase in BMP value and also in methane production kinetics, using a high I/S ratio, thus preventing any inhibition phenomenon. BMP curves from the tested samples were summarized in fig. 4.10, while methane production rate from the tests was reported in fig. 4.11.

From fig. 4.10, it could be highlighted that BMP value was practically coincident using I/S=3 (217.9 NmL CH_4/g COD_{added}) and I/S=5 (224.0 NmL CH_4/g COD_{added}), even if the latter showed a faster kinetics; instead, a consistent increase in BMP value was obtained from I/S=5 to I/S=8 (360.4 NmL CH_4/g COD_{added}). Finally, as for sonication pre-treatment, a slight increase in BMP value was observed (369.7 NmL CH_4/g COD_{added}), that merely represented an increase of 2.6%, if compared with untreated whey; obviously, this increase

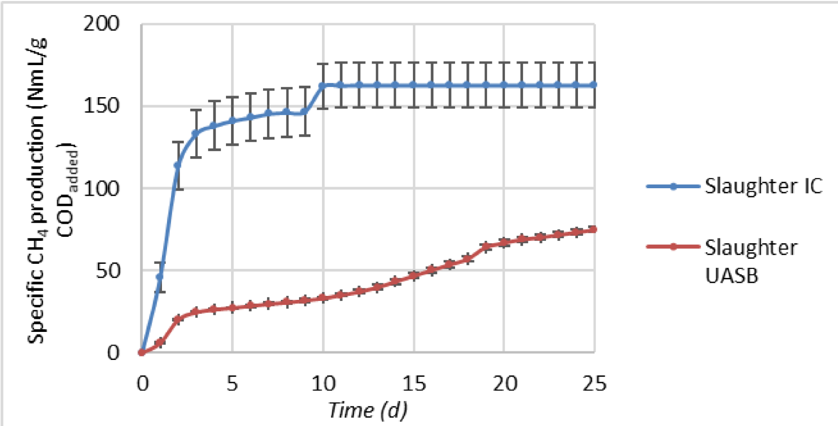

Figure 4.9: Comparison between BMP test results on slaughterhouse waste, using Tolmezzo (UASB) and Castelfranco (IC) biomasses as inocula

cannot be sufficient to justify energy expenses for US pre-treatment. A detailed analysis of the time series of untreated and sonicated whey showed that sonication augmented methane production in a more consistent way in the first days of test, showing a maximum at day 5, where an increase of 16.4% was registered, if compared to untreated whey CH_4 production. As a consequence, a further series of tests was planned and executed (paragraph 4.3.4), using different US conditions, in terms of US power and energy, to study the influence of these parameters on methane yields in the AD process.

4.3.4 Analysis of methane fluxes

The analysis of the fluxes, beside cumulative CH_4 production, is also significant, because it can be useful to identify when methane production is more intense, obtaining valuable information on the biodegradability, and also on the best operating conditions (in terms of HRT) that should be used in full-scale reactors.

Fig. 4.11 reports, as an example, CH_4 production flux from CW tests (at different I/S ratios), that was simply obtained by calculating the derivative of CH_4 production curves (shown in fig. 4.10). It could be seen that, for all the tests, two distinct peaks appeared, the first on day 1 of digestion, and the second between day 5 and 7. This probably indicated the degradation of two types of

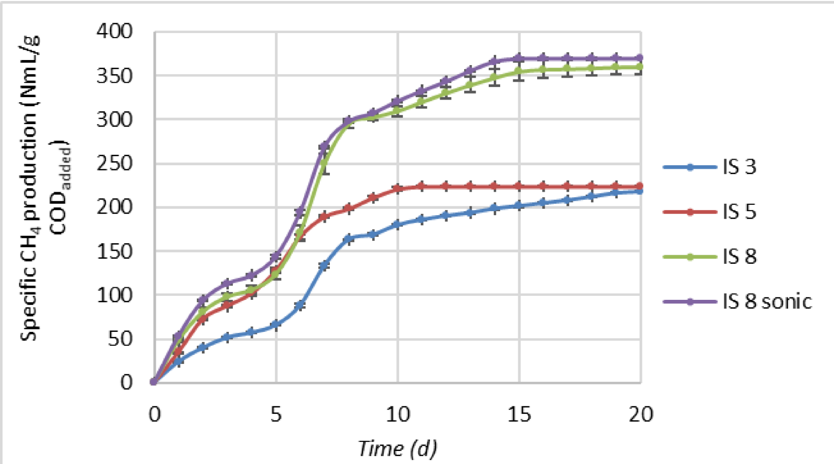

Figure 4.10: Comparison between BMP test results on CW, using different I/S ratios

compounds, the first peak being associated with readily biodegradable molecules, and the second being related to the degradation of slowly degradable compounds. Methane production rate was particularly intense in this second peak, for the samples tested at I/S ratio of 8.

The analysis on specific CH_4 fluxes was extended to all the selected matrices; given the high activity of this biomass, the results obtained using Castelfranco IC biomass were used for the successive elaborations. In particular, tab. 4.1 reports the specific methane production peak of the different substrates (expressed as NmL CH_4/g COD_{added} day), and the corresponding digestion time.

It can be seen that condensate water produced the highest peak (210.4 NmL CH_4/g COD_{added}day), that happened, in addition, during the first digestion day, probably due to the fact that the IC granular biomass was highly adapted to this substrate, differently from the other substrates. For all the other matrices, in fact, the peak was lower, and it appeared on the second day of digestion, as for OFMSW leachate and slaughterhouse waste, while, as previously described, it appeared between day 5 and 7, in CW digestion. From the quantitative point of view, all the substrates, except from condensate water, had a comparable specific CH_4 peak, in the range of 40.0-78.8 NmL CH_4/g COD_{added}.

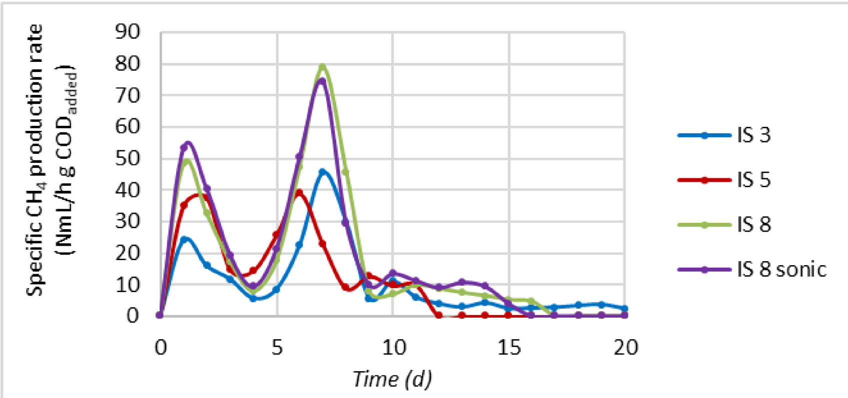

Figure 4.11: Comparison between specific CH_4 production rates of CW using different I/S ratios

Table 4.1: Specific CH_4 peak from selected substrates, and corresponding digestion time

Substrate	Spec. CH_4 peak (NmL/g COD_{added}d)	Time (d)
CW (I/S=3)	45.4	6
CW (I/S=5)	38.8	5
CW (I/S=8)	78.8	6
Sonic CW (I/S=8)	74.3	7
Condensate water	210.4	1
OFMSW leachate	40.0	2
Slaughterhouse waste	67.6	2

4.3.5 BMP tests on sonicated CW

As previously mentioned, a further series of tests was conducted, to better evaluate the feasibility of US pre-treatment to increase CH_4 production from CW. Two different whey streams (FW, fat whey, and SW, skimmed whey) were selected, and different sonication conditions, in terms of power and treatment time, were tested. Input parameters and observed increase in CH_4 yields were summarized in tab. 4.2, where also theoretical energy gain from surplus in BMP value was calculated. In this set of tests, all the results were expressed on VS basis, in order to compare in a simple way the obtained results with literature references.

The results, reported in tab. 4.2, highlighted that the best outcomes, in terms of BMP increase, were obtained at low US power (40 W) and time (5 min), for both kinds of CW: the successive increase in US energy, applied for longer times (10 min) or using higher power (80 W), did not translated into a correspondent augmentation of methane yields. In fact, all the tests executed at high US energy (in the range of 502.8-1,387.5 Wh/kg VS) showed a negative final energetic balance. In particular, at the same US energy, it appears advantageous, from these data, to prefer higher US power, rather than longer US time: at the same applied US energy, a significant increase (up to 16.0%) was registered for samples treated at 80 W power for 5 min, while even a reduction in final BMP (until -5.7%) was encountered when using 40 W power for 10 minutes.

Net energy gain could be calculated as the difference between increase in BMP yield and US energy expense. As for the difference between different kinds of CW, a similar behaviour in BMP curves was observed in fat and skimmed whey: a significant increase in CH_4 production (up to 53%) was registered after 2 days of digestion, particularly evident in the case of 5 min US pre-treatment; after that, this increase progressively reduced, until the final BMP was reached. However, as can be visible from the graphs reported in fig. 4.14 and fig. 4.16, the obtained increase in BMP value, and methane production kinetics, was more visible when testing fat whey, instead of skimmed whey.

It could be concluded that an effective increase in final BMP from sonicated whey was observed only from the tests executed at US time of 5 minutes, and it was around 15%; interestingly, even a slight reduction in BMP value was observed in the tests where a 10 min US pre-treatment was applied. Thus, a strong non-linear correlation between applied US energy and increase in BMP yield was found. These findings suggest that US can be useful to accelerate AD process, disaggregating large molecules in simpler ones, but operating conditions should be properly set, in order not to vanish US effect; final methane yield,

Table 4.2: Input parameters for CW sonication tests and obtained increase in CH_4 yields in BMP tests

Matrix	US P (W)	US t (min)	Spec US EE (Wh/kg VS)	CH_4 increase (NmL/g VS)	Theor en gain (Wh/kg VS)
FW	40	5	251.4	70.6	786.1
FW	80	5	502.8	68.7	747.1
FW	40	10	502.8	-24.6	-268.0
FW	80	10	1,005.5	-6.7	-72.5
SW	40	5	346.9	38.8	422.5
SW	80	5	693.7	30.2	328.9
SW	40	10	693.7	-14.0	-152.4
SW	80	10	1,387.5	-18.5	-201.0

Figure 4.12: CW sonication

obtainable from CW, can be augmented at least by 15%, using this pre-treatment.

4.3.6 BMP tests on brewery waste

Brewery waste was tested using Udine AD sludge as inoculum, because of the general high solid content of the material; I/S ratio was set at 3 (on VS basis), as for BSG (trub), while, according to some preliminary tests, a higher I/S ratio of 6 (on VS basis) was adopted for spent yeast, whirlpool residue and end-of-fermentation beer. The results were expressed on VS basis, as well, given the impossibility of measuring tCOD for most of the samples, and also to fruitfully compare the obtained results with literature evidences.

The available organic streams from a selected local brewery, namely trub, spent yeast, whirlpool residue and end-of-fermentation beer, were separately tested, to establish methane potential of each fraction. The results were summarized in fig. 4.17, and highlighted a substantial biodegradability of all the fractions. However, a remarkable aspect was the fact that spent beer, that had the lower solids content between all brewery residues, gave the highest CH_4 production in the first digestion day, while, successively, methane production was substantially inhibited (with a low final BMP of 126.0 NmL CH_4/g VS_{added}); the

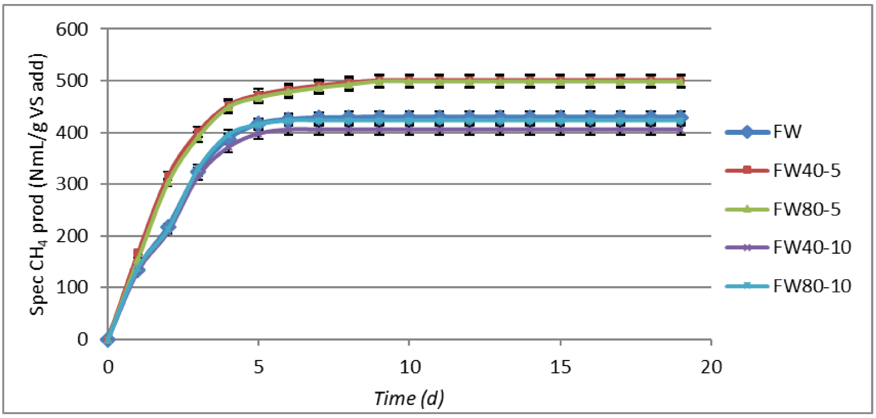

Figure 4.13: CH_4 production from US tests on fat CW

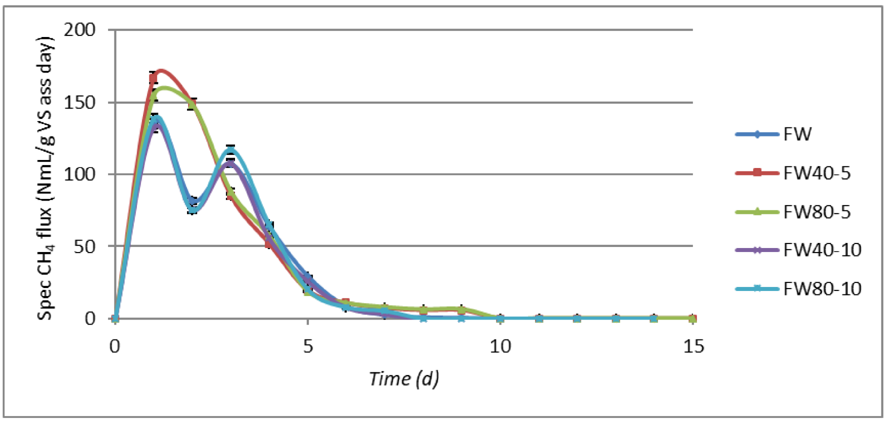

Figure 4.14: CH_4 flux from US tests on fat CW

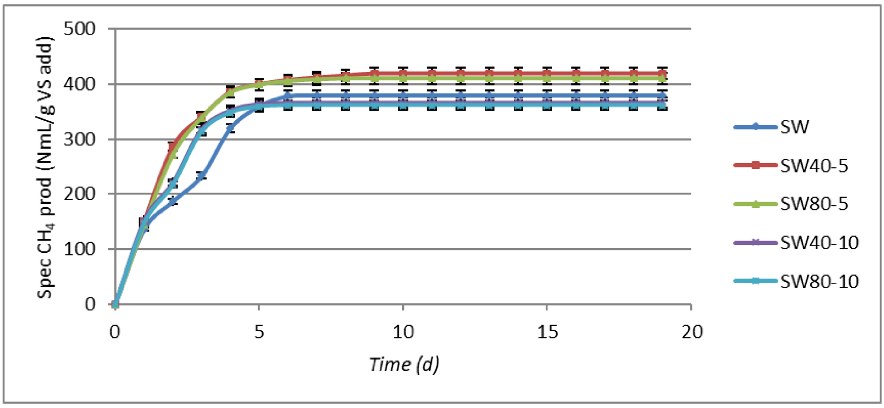

Figure 4.15: CH_4 production from US tests on skimmed CW

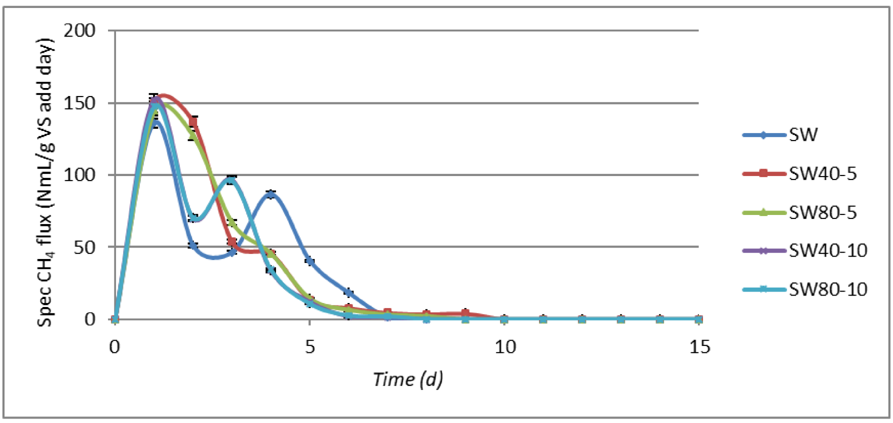

Figure 4.16: CH_4 flux from US tests on skimmed CW

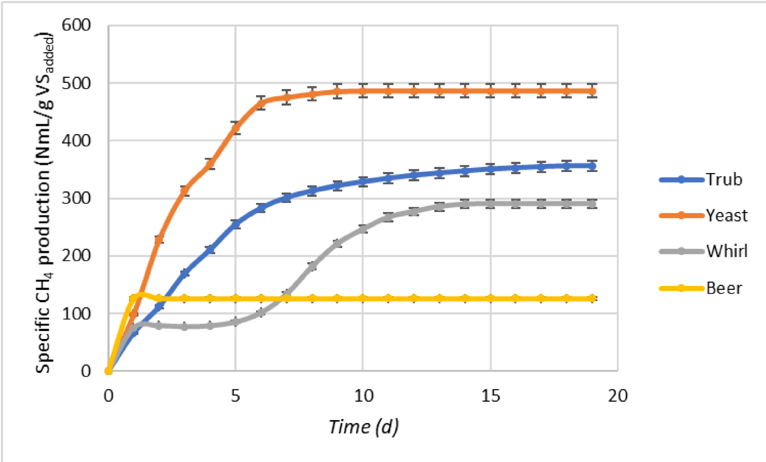

Figure 4.17: BMP tests results on brewery waste (trub, yeast, whirlpool residue and end-of-fermentation beer)

highest BMP value, instead, was obtained from spent yeast (486.9 NmL CH_4/g VS_{added}), while lower BMP was found both for trub (356.1 NmL CH_4/g VS_{added}) and whirlpool residue (290.3 NmL CH_4/g VS_{added}). In fig. 4.18, CH_4 fluxes from brewery waste tests were depicted: again, a strong initial peak both for end-of-fermentation beer and yeast emerged, while trub, and mostly whirlpool residue, had significant methane production peaks from day 4 to day 7.

Effect of biochar on BMP yield

Biochar (fig. 4.19) is a carbonaceous material, formed under combustion of plant materials in low-zero oxygen conditions; currently, biochar has been tested to improve the soil ecosystem, digestate quality and, more recently, AD process [165]. There is a growing interest, in particular, in using biochar in AD, to both increase the recovery rate of the process during substrate-induced inhibition and decrease the nutrient loss before and after land application [166].

In this work, biochar was tested, together with granular activated carbon (GAC), on selected brewery samples, in order to evaluate a possible increase in CH_4 yield in BMP tests. In particular, spent yeast and whirlpool residue were tested, at the same operating conditions described in 4.3.6. Biochar and GAC

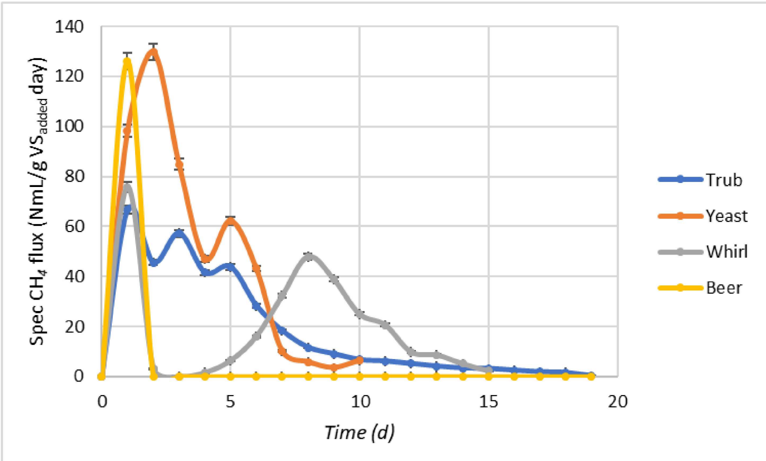

Figure 4.18: CH_4 production rate from brewery residues

Figure 4.19: Biochar [167]

Table 4.3: Biochar characterization

Parameter	Value
Bulk density (kg/m^3	446
Specific surface area BET (m^2/g)	327
Total water (% w/w)	2.6
Ash content at 550 °C (% w/w)	6.1
Gross calorific value (kJ/kg)	30,875
H (% w/w)	1.54
C (% w/w)	1.54
N (% w/w)	1.54
O (% w/w)	1.54
H/C ratio	0.21
O/C ratio	0.01
pH	8.4
Electrical conductivity μS/cm	217

dosage was set at 0.2 g/g VS_{sub}, following the indications of [168].

Commercial GAC was obtained by Sigma-Aldrich, having particle size of 50-150 μm; biochar, instead, was furnished by University laboratory, and some meaningful characterization parameters were reported in tab. 4.3.

The results from BMP tests, reported in fig. 4.20 and fig. 4.21, highlighted a significant increase in biogas yield from brewery residues, both with the addition of GAC and biochar. This effect was similar between spent yeast and whirlpool residue; in particular, BMP value of yeast increased up to 641.0-642.2 NL CH_4/kg VS_{added} (+31.7%, compared to untreated yeast), while BMP from whirlpool residue rose up to 404.4 NL CH_4/kg VS_{added} (+39.7%, if compared to untreated whirlpool residue).

This strong effect can be ascribed to a significant improvement in C/N ratio, as for spent yeast, where a high N concentration emerged (Chapter 3), that allowed a better operation of anaerobic bacteria, while, as for whirlpool residue, this meaningful increase can be explained with peculiar biochar characteristics, that is characterized by a porous morphology and surface crevices, providing ideal conditions for microorganisms adhesion. This led to an enhanced digestibility and also help to shorten the digestion start-up time; a significant effect was indeed seen in the second methane peak, that appeared after 7-8 days of digestion,

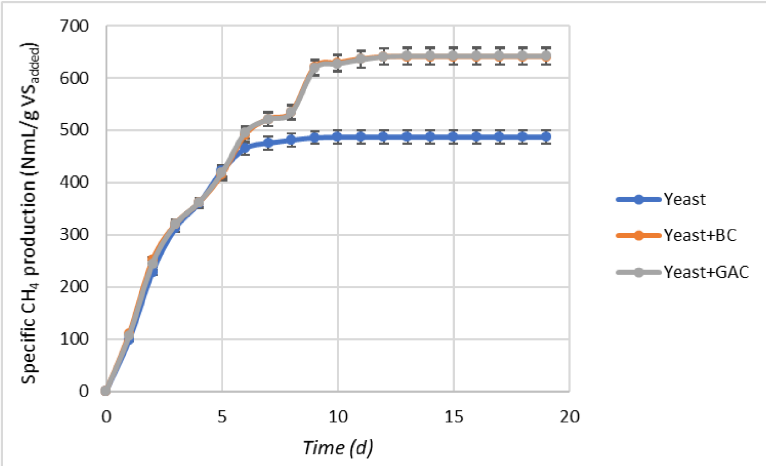

Figure 4.20: Effect of BC and GAC on yeast BMP curves

and was consistently more pronounced when adding biochar, if compared to untreated whirlpool residue.

From these basic results, it could be concluded that the use of low-cost biochar, coming, as an example, from local biomass combustion plants, should be encouraged as an additive in AD processes, in order to increase methane yields, when testing brewery residues. The integration of thermochemical processes with AD represents, in fact, an interesting strategic approach towards a sustainable resources conversion and management ([169]; this integration, in fact, could potentially expand the range of available feedstocks to biologically recalcitrant substrates, such as paper and woody materials, leading to the development of local sustainable circular economies [170].

4.3.7 Discussion

A comparison with literature BMP tests on similar substrates can be fruitful: as for OFMSW leachate, in the work by Bolzonella et al. [171], a 67% VS extraction from OFMSW was obtained after 4.5 days digestion, using waste-to-water ratio of 1:8 (lower than that used in the actual study). They observed 2 separate peaks in BMP curves: the first, from the starting of the tests until day 3, was related to the conversion of easily biodegradable substances, while

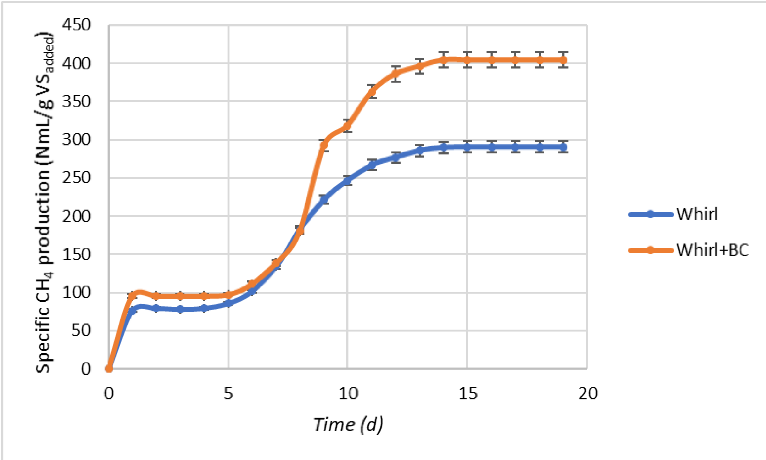

Figure 4.21: Effect of BC on whirlpool BMP curves

the second, from day 3 to day 10, corresponded to the degradation of slowly degradable molecules. Similarly to what happened in the actual tests, both using Tolmezzo and Castelfranco inocula, CH_4 generation interrupted after 10-12 days of digestion, indicating that OFMSW leachate is a substrate characterized by good biodegradability, and it does not require long digestion times to be fully degraded.

As for CW, Erguder et al. [150] reported, in some of the reactor tests, CH_4 production inhibition, similarly to what happened in this work, when using a low I/S ratio of 2. Moreover, they measured a maximum methane generation of 424 NmL CH_4/g COD, or 23.4 L CH_4/L whey. Labatut et al. [172] reported methane yield from a great variety of substrates; in particular, they claimed a CH_4 yield of 423.6 NL CH_4/kg VS_{added} for CW. Due to acidification, the first actual tests on CW, at I/S=2, showed a scarce methane yield of 41.9 NL CH_4/kg VS_{added}. From the tests executed at higher I/S ratios, instead, BMP value from this substrate increased until 360.4 NmL CH_4/g COD_{added}, corresponding to 529.0 NmL CH_4/g VS_{added}, that was significantly higher than what reported in [150].

As for US tests on CW, limited work was found in literature regarding this pre-treatment on dairy substrates. In [173], it was underlined a good increase in biogas production and methane content from US pre-treated CW, together with the observation that larger doses of applied US energy did not corresponded to a proportional increase in methane production. This was similar to what obtained in this work, where a clear correlation between applied US energy and increase in BMP yield was not found. Similar results were obtained in [174], where a wide range of US power (22, 44, 66, 88, 110 W) and time (6-45 min) were used to pre-treat landfill leachate, before AD process. They reported a maximum CH_4 production at 44W power, and a decreasing CH_4 yield at higher US power. It was observed that transient cavitation, occurring at high power inputs, could accelerate biosolids solubilization; however, organic substrates, produced from this pre-treatment, were not easily consumed by microbial cultures [174].

Interestingly, differently from what obtained in this work, a linear correlation between applied US energy and methane yield was observed in [175], where microalgal biomass was tested as substrate: using a US energy in the range of 16-67 MJ/kg TS, they obtained a linear increase in BMP of 20-30%. Another remarkable work reported that US pre-treatment of municipal waste activated sludge, with applied US energy in the range of 1,000-10,000 kJ/kg TSS, led to an increase in CH_4 production in the range of 15-24% [176].

As for condensate water, in the work by Meyer and Edwards [177], the physicochemical characteristics and BMP yields of a great variety of P&P WWs were reported; in particular, as for kraft condensate, a broad COD removal range of 41-68% was reported, together with a methane yield of 0.32 m^3 CH_4/kg $COD_{removed}$. Considering the maximum removal rate of 68%, a specific methane yield of 0.22 m^3 CH_4/kg $COD_{removed}$ was calculated, that was coherent with the BMP measured in the tests executed using Tolmezzo sludge. Actually, a significantly higher CH_4 production was obtained in the tests performed using Castelfranco sludge (687 NmL CH_4/g COD_{added}).

As for brewery waste, in [178] BMP from BSG was reported to be 545 L CH_4/kg TS, that actually was significantly higher than the obtained BMP from trub (356.1 L CH_4/kg VS, corresponding to 345.4 L CH_4/kg TS). However, also from this work it could be highlighted that trub characteristics are highly variable; in particular humidity, and consequently TS and VS, can vary in a broad range, depending on a better or worse separation of the liquid content, at the end of the process.

As for spent yeast, a part from the high methane production, that was found in this tests, a synergistic effect with anaerobic bacteria should be evaluated (as was done, for example, in [179]), when co-digesting the different brewery residues. Because of the limited amount of time, it was not possible to perform these tests, but this can be a suggestion for a successive work. It was observed that adding a carbon-rich matrix, such as biochar, a significant increase in methane yield can be obtained, both from spent yeast and whirlpool residue.

Finally, as for slaughterhouse waste, BMP tests reported in [126] showed an extreme variability (until extremely high values of 815 NmL CH_4/g VS_{added}), and ABP2, that was considered in Chapter 3 the substrate most comparable to the analysed waste, actually produced the lowest yield of 117 NmL CH_4/g VS_{added}. In the tests executed using Tolmezzo sludge, the actual waste produced a BMP yield of 150.2 NmL CH_4/g VS_{added}, slightly higher than ABP2 (considering a VS basis). The second set of tests, instead, produced a BMP yield of 326.8 NmL CH_4/g VS_{added}, that was similar to the BMP of SH5 (coming from animal intestines), reported in [126], that was 344 NmL CH_4/g VS_{added}.

4.4 Kinetic parameters estimation

As could be inferred from the theoretical deepening and the results presentation in this chapter, the experimental determination of BMP value is a time-consuming process (each test requires approximately 20-25 days), and thus it is not always a practically feasible management tool at industrial scale, for AD optimization or implementation. Therefore, it is attractive to use faster methods to predict how much methane gas can be produced from a given substrate. This is particularly true when making theoretical studies without access to laboratory facilities, or when a fast prediction of the BMP from new substrates is required [180].

Due to the microbial role in the anaerobic process, kinetic models were commonly applied to simulate anaerobic biodegradation. Similarly to bacterial growth phases, biogas production shows a rising curve, and a successive decreasing curve, indicated by exponential and linear equations [181]. Understanding the kinetics of methane production from feedstocks is important for designing and evaluating anaerobic digesters operations. The first order kinetic model, Gompertz equation and Chen and Hashimoto models have been successfully applied to anaerobic treatment, using different kind of reactors [182].

4.4.1 First order kinetic model

The first order kinetic model is the simplest model, but it does not predict the conditions for maximum biological activity and possible system failures. Hydrolysis is often assumed to be the rate-limiting step in AD and, based on this consideration, researchers modelled batch BMP data using first-order hydrolysis models, to obtain valuable information about hydrolysis kinetics. A basic first order equation can be written as follows, where k is the disintegration rate constant and C is the biodegradable substrate concentration (expressed as VS or COD) [180]:

$$\frac{dC}{dt} = -kC \tag{4.1}$$

Rearranging and integrating for time *t=0* to *t* days gives:

$$\frac{C_t}{C_0} = e^{-kt} \tag{4.2}$$

However, it is easier to derive the model by using the gas measurement instead of measuring C, which is difficult. The relationship between VS (or COD) and methane production can be exemplified as follows, where G(t) is the cumulative methane yield at digestion time t (evaluated in mL/g COD or VS) and G_0 is the methane potential of the substrate (mL/g COD or VS) [180]:

$$\frac{C_t}{C_0} = \frac{G_0 - G_t}{G_0} \tag{4.3}$$

Substituting eq. 4.3 in eq. 4.2, the general expression of methane evolution in the AD process can be obtained. The constant *k* represents methane production rate constant, and is assumed to be the hydrolysis constant [180].

$$G(t) = G_0(1 - e^{-kt}) \tag{4.4}$$

Eq. 4.4 is an accurate representation of the BMP results when:

1. Hydrolysis is the rate-limiting step;
2. G_0 represents the total yield of hydrolysable VS or COD at the beginning of the tests [183].

Considering the analysed substrates, that can be modelled as complex and heterogeneous matrices, the hypothesis of hydrolysis as the rate-limiting step is well respected, so a data regression could be effectively made from selected BMP tests, to obtain the hydrolysis constant of AD process; input data were CH_4 production curves and the final BMP value, that was taken as G_0.

In particular, the tests made with IC granular biomass were considered, as for CW (at I/S=3, 5, 8), sonicated CW, OFMSW ground leachate, condensate water and slaughterhouse waste, while, as for brewery waste (trub, spent yeast and whirlpool residue), the results reported in paragraph 4.3.5 were used. Hydrolysis constant k was chosen in order to maximize R^2 between measured and predicted data.

4.4.2 Modified Gompertz equation

The Gompertz model was originally developed to fit human mortality data, and was set on an exponential relationship between specific growth rate and population density. Gibson et al. [184] modified this model to a function that describes cell density during bacterial growth periods, in terms of exponential growth rates and lag phase duration.

The assumption of correspondence between methane production rate (in a batch digester) and the specific growth rate of methanogenic bacteria led to the following equation, where R_{max} expresses the maximum methane production rate (mL/g COD day) and λ is lag phase duration (day).

$$G(t) = G_0 exp\left[-exp\left[\frac{R_{max}e}{G_0}(\lambda - t) + 1\right]\right] \tag{4.5}$$

The lag phase represents the minimum time taken to produce biogas, or necessary for environmental acclimation of bacteria, while R_{max} describes specific growth rate of methanogenic bacteria. Also modified-Gompertz model was used to simulate the selected BMP tests, and in this case R_{max} was calculated from the time series of CH_4 fluxes, while λ was conveniently chosen to obtain the best fitting with the experimental data, observing the initial progression of the curves and maximizing R^2 between predicted and measured methane yields.

4.4.3 Kinetic models application

For each of the analysed substrates, a comparison between measured and predicted BMP data by applying first-order model and Gompertz equation was made. In particular, measured CH_4 production from BMP tests was correlated with predicted CH_4 production, and the R^2 value was calculated for each of the selected matrices; in addition, hydrolysis constant, obtained from first order model application, was calculated, as well as lag phase duration, as for Gompertz model. The results were schematically summarized in tab. 4.4.

It could be seen that a better prediction was generally obtained applying kinetic model, except for CW (at lower I/S) and brewery spent yeast, where the tests were better fitted by Gompertz equation. Inferred hydrolysis constants were in the range of 0.12-0.21 d^{-1} in CW, as low as 0.05 d^{-1} in condensate water, in the range of 0.23-0.36 d^{-1} in brewery waste and as high as 0.48 d^{-1} in slaughterhouse waste. Lag phase duration, instead, was in the range of 1.5-4 days in CW, 5 days in OFMSW leachate, 9 days in condensate water, 0.2 days in slaughterouse

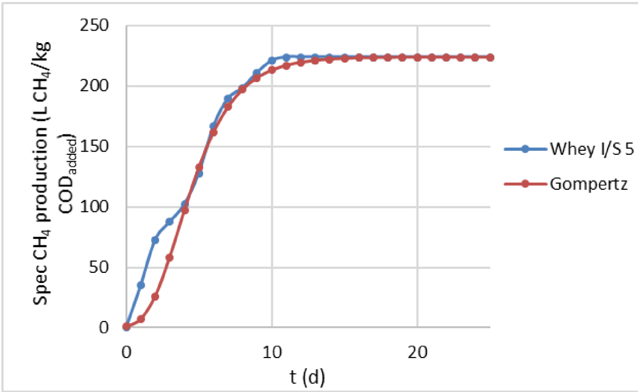

Figure 4.22: Specific CH_4 production from BMP tests on CW at I/S=5: comparison between effected and simulated data (Gompertz equation)

waste, in the range of 0.4-0.6 days in brewery yeast and trub, as high as 5.9 days in brewery whirlpool residue.

In general, as could be expected, it was seen that Gompertz equation did not allow to predict CH_4 curve evolution in the first digestion days, where an intense CH_4 production was generally encountered from each substrate, because of the introduction of the lag time λ, which, on the other hand, was necessary to obtain a good fitting in final BMP value. This observations can be also visualized in fig. 4.22, where the modelled curve was compared with the real experimental curve, as for CW (at I/S=5). In the successive graphs (fig. 4.23 and fig. 4.24), the comparison between predicted and measured CH_4 production was reported, both for Gompertz equation and kinetic model.

This simple approach, anyway, can be useful in comparing substrates behaviour in anaerobic environment; it is an approximate method, that requires, as input data, only methane production curves (directly obtainable from AMPTS equipment) and does not consider even COD or VS removal. As described in Chapter 2, more complex and complete approaches should be used for AD modelling, to get more in depth analysis of the process and to understand the influence of a variety of operating parameters on process efficiency and biogas production. Most of them, like ADM1 model, ask for a detailed influent characterization, as well as stoichiometric and kinetic parameters. Given the limited data availability for this work, however, it was not possible to further deepen

Table 4.4: Comparison between R^2 value obtained with first-order and Gompertz models application and obtained hydrolysis constant and lag phase duration

Matrix	R^2 (first-order)	k (hydrolysis constant) (d^{-1})	R^2 (Gompertz)	Lag phase, λ (d)
CW (I/S=3)	0.9651	0.12	**0.9736**	4
CW (I/S=5)	0.9742	0.21	**0.9843**	1.5
CW (I/S=8)	**0.9585**	0.14	0.9567	3
Sonic CW	**0.9671**	0.15	0.9617	2.7
OFMSW leach	**0.9761**	0.12	0.8226	5
Condensate water	**0.9480**	0.05	0.8807	9
Slaughter waste	**0.9707**	0.48	0.9603	0.2
Brewery trub	**0.9956**	0.23	0.9905	0.6
Brewery yeast	0.9892	0.35	**0.9931**	0.4
Brewery whirlpool	**0.9887**	0.36	0.9704	5.9

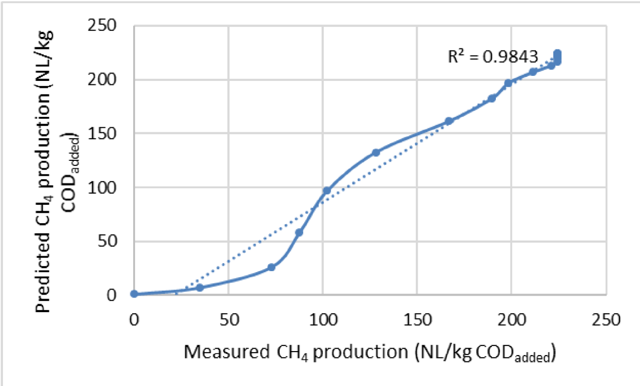

Figure 4.23: Linear interpolation between measured and predicted CH_4 yield (using Gompertz equation) and R^2 value (substrate: CW, I/S=5)

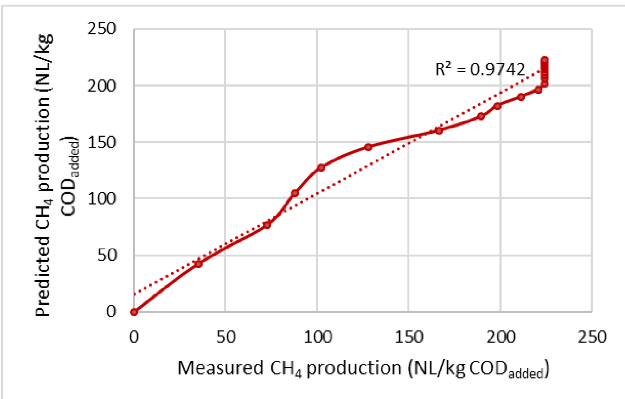

Figure 4.24: Linear interpolation between measured and predicted CH_4 yield (using kinetic model) and R^2 value (substrate: CW, I/S=5)

this topic.

4.5 Conclusions

BMP tests were performed on selected substrates (CW, condensate P&P wastewater, OFMSW leachate, slaughterhouse waste, brewery waste) as a useful tool, to get reliable information about obtainable CH_4 yields and biodegradability in AD processes. This chapter was aimed at introducing the continuous UASB tests, that will be presented in Chapter 5, and that were performed on some of the selected substrates, namely CW, condensate P&P wastewater and OFMSW leachate.

BMP tests were conducted, in particular, using three different biomasses, the first (granular type) coming from existing Tolmezzo UASB plant (inactive since 10 years), the second (highly active, again granular sludge) was withdrawn from a full-scale IC reactor, and the third (flocculent sludge) was taken from Udine WWTP anaerobic digester. A comparison between the obtained results was done, and some meaningful considerations were made; in particular, it was seen that a very high methane production was registered for condensate water, using the highly active Castelfranco biomass, because of the fact that it was already adapt to treat this kind of substrate.

Summarizing, some general considerations could be made on the biodegradability characteristics of the selected substrates:

- CW, at an I/S ratio of 2, produced a sudden acidification in BMP tests, both with the more active and the low activity biomasses, so higher I/S ratios (3, 5, 8) were tested. A substantial increase in specific CH_4 production was obtained from I/S=5 to I/S=8 (the maximum BMP of 369.7 NmL CH_4/g COD_{added} was registered at I/S=8); this suggested to properly dilute this matrix in continuous tests, verifying not to overload the reactor, given also the extremely high COD concentration of this matrix;

- Sonication pre-treatment was tested on CW, and it led to a moderate increase in CH_4 yield (around 15%); in particular, a higher methane production kinetics (up to 43% increase after three days of digestion) was registered in the first digestion days. A strong non-linear correlation between applied US energy and increase in BMP yield was found: the tests executed with a US treatment time of 5 min gave significantly better results than that performed using US treatment time of 10 min, where no effect was visible in methane yield;

- Condensate water, in the case of low activity granular biomass, required long digestion time, to achieve final BMP value, while, using the highly active granular biomass, it was readily digested, giving the highest methane yield from the analysed substrates (687 NmL CH_4/g COD_{added}), so it could be suggested to forecast a proper acclimation period, before starting the operations, when using a non-adapted biomass;

- OFMSW leachate produced moderate methane yields (final BMP of 216.5 NmL CH_4/g COD_{added}), with relatively fast kinetics, even with the low activity biomass; however, from successive experimental trials (that will be reported in Chapter 5) it was highlighted that the physicochemical characteristics of the obtained leachate strongly depended on the geometrical characteristics of leaching bed and on the main operating parameters, such as waste characteristics and origin, waste-to-water ratio, contact time, particle dimensions, so it is not very convenient to scale-up such percolation systems;

- Slaughterhouse waste produced low methane values, particularly when the low activity granular biomass was employed; however, also the use of the more active biomass did not give a high final BMP value (162.8 NmL CH_4/g COD_{added}), indicating a general low biodegradability of this matrix. Indeed, it was decided not to further investigate this matrix, in the successive continuous phase;

- Brewery waste, that was tested with Udine anaerobic sludge, showed different biodegradability behaviour: spent yeast gave consistent CH_4 yields (BMP up to 486.9 NmL CH_4/g VS_{added}), while trub (BMP of 356.1 NmL CH_4/g VS_{added}), whirlpool residue (BMP of 290.3 NmL CH_4/g VS_{added}) and, mostly, end-of-fermentation beer (BMP of 126.0 NmL CH_4/g VS_{added}), produced less methane;

- GAC and biochar addition in spent yeast and whirlpool BMP tests led to a significant increase in methane production, in the range of 32-40%, thus a synergistic effect with local biomass should be deepened, to follow circular economy pattern. In addition, a possible improvement of the single digestion process could be co-digestion of all these brewery matrices, given also the complementary characteristics, that were observed in the characterization phase.

Finally, a simplified kinetic analysis was carried out on BMP tests results, using first-order kinetic model and modified Gompertz equation, and, in particular, simplified hydrolysis constants and lag-phase durations were obtained.

Chapter 5

Continuous UASB tests

Continuous tests were performed on a pilot-UASB reactor, that was realized and run in Tolmezzo WWTP, and were aimed at verifying the feasibility of UASB treatment of the most suitable substrates, among the analysed ones; in particular, UASB tests were conducted on CW, condensate water and OFMSW leachate.

5.1 Materials and methods

The general scheme of the installed reactor was reported in fig. 5.1, while a photograph of the realized pilot unit was shown in fig. 5.2. It consisted of:

- Influent storage tank (V=1,000 L), that allowed the influent storage for a period of at least 2 weeks, depending on the HRT used in UASB reactor;

- Pre-acidificator (V=40 L), fed by a first peristaltic pump, which had the function to heat the influent to the proper mesophilic temperature (35 °C) and correct pH to the set-point value (6.7);

- UASB column (V=65 L), fed by a second peristaltic pump, where highly active granular sludge, taken from Castelfranco IC reactor (also used in the BMP tests, reported in Chapter 4), was introduced. As described in Chapter 2, where UASB reactors configuration was introduced, the influent was fed at the base and flew upwards, while the effluent was separated from biogas in the three-phase separator;

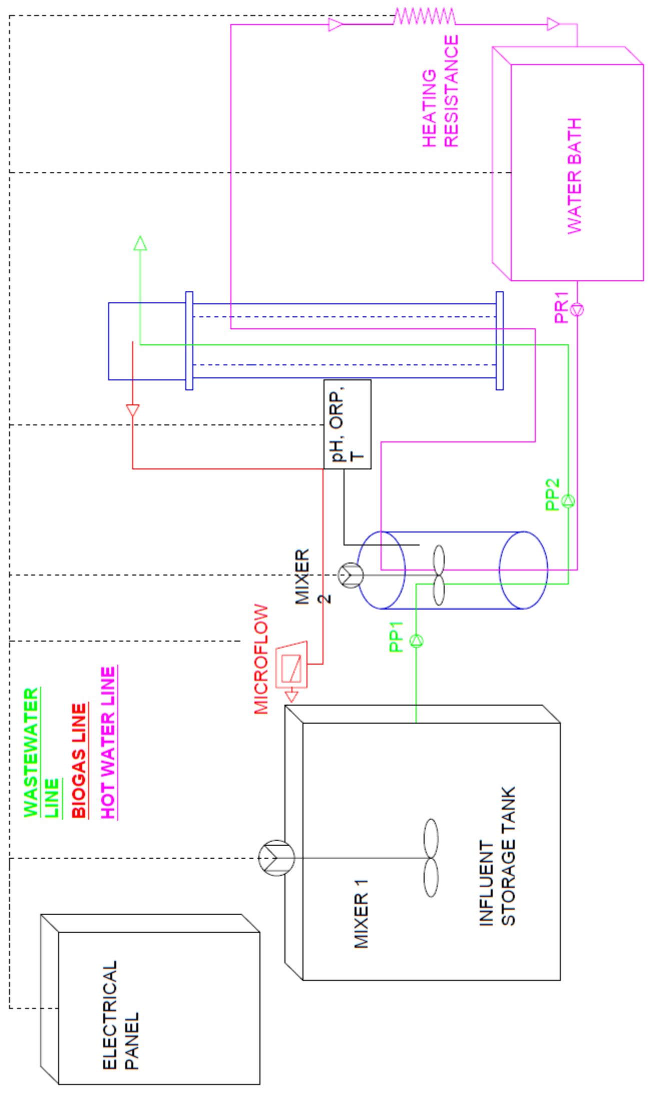

Figure 5.1: Pilot UASB reactor process scheme

- Biogas line, composed of an accumulation headspace and a biogas registration unit (μ-*Flow*, Bioprocess);
- Water heating bath and recirculation pump, with T set-point (fixed at 35 °C, coherently with the temperature used in BMP tests), which heated both the pre-acidificator and the UASB column to the desired mesophilic T;
- 2 mixers, one for influent storage tank and one for pre-acidificator homogeneization;
- Leaching bed (fig. 5.3) and waste shredder, for the tests executed on OFMSW.

The tests were executed on CW, condensate water and OFMSW leachate, and the operating conditions were properly set for each substrate, considering the results obtained from physicochemical characterization (Chapter 3) and BMP tests (Chapter 4). Moreover, effluent recirculation was introduced, in particular for the tests on condensate water (to simulate full-scale operations, and reduce soda consumption for pH correction) and OFMSW leachate (because of the limited amount of available leachate). Given the fact that biogas separation in its constituting components (mainly CH_4 and CO_2) was not possible, according to a preliminary literature evaluation, it was estimated a CH_4 percentage in biogas of 70%.

In the following paragraphs, the main results from each substrate tests will be highlighted, together with some considerations for eventual successive studies.

5.2 CW tests

The tests were conducted on a mixture of first (50%) and second (50%) CW, in order to have a significant mixture, representative of the real stream produced by local dairies; following the results of laboratory physicochemical analysis (Chapter 3) and BMP tests (Chapter 4), whey was properly diluted with tap water, in a proportion of 1:50 v/v, to obtain a COD concentration that could be easily treated in the start-up phase of the pilot-reactor. Whey dilution was basically intended not to overload the reactor, and to allow granular biomass to adapt to this substrate. It could be forecast, in longer pilot-tests, to progressively reduce this dilution, in order to increase influent COD, OLR and, consequently, also methane production.

Figure 5.2: Pilot UASB reactor, located in Tolmezzo WWTP

As for the main operating parameters, influent flowrate was fixed to 36 L/day, corresponding to a mean OLR of 0.81 kg COD/m^3day. Up-flow velocity, v_{up}, was calculated as 0.055 m/h; HRT was set at 40 h.

The results from a period of 1 month tests were reported in fig. 5.4: it was observed a good adaptation of granular sludge to diluted whey, with high COD removal (mean 84.6%), despite of the natural fluctuations in influent load, that occurred due to the high heterogeneity of the substrate. Mean COD concentration in the influent was 1.31±0.49 g/L, while mean effluent COD was 0.18±0.09 g/L. Mean biogas production was 148.5 L CH_4/kg $COD_{removed}$. It should be pointed out that, due to some operational problems, actually only punctual biogas production data were available, so a defined trend in biogas production could not be obtained.

A comparison of the obtained results with literature evidences can be fruitful: Rico et al. [185] studied co-digestion of CW with manure, with a HRT of 2.2 days and a high OLR (up to 19.4 kg COD/m^3d), and they reported a stable operation until a CW fraction in the feed of 75%; in this conditions, they claimed a methane production up to 6.4 m^3 CH_4/m^3d. Moreover, using a lower CW fraction of 60%, OLR could be increased up to 28.7 kg COD/m^3d, with a reduced HRT of 1.3 days. Erguder et al. [150], instead, treated undiluted CW in a two-stage UASB

Figure 5.3: OFMSW leaching bed

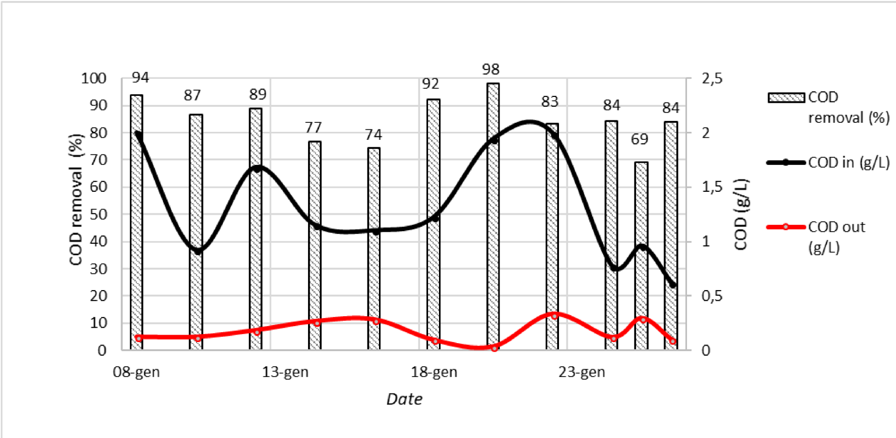

Figure 5.4: Results from UASB tests on CW

reactor, and they concluded that low HRT, down to 2 days, can be used even in single-stage UASB reactors, but alkalinity and nutrients should be supplied, in order to allow stable operations.

It could be concluded that the feasibility of continuous tests using CW as a feed had been already proved in literature; a longer pilot-test campaign should be forecast, however, to underline the specific limit of the system (in terms of OLR), as well as the maximum CH_4 production obtainable in continuous operations.

5.3 Condensate water tests

As for condensate water tests, it was tried to reproduce full-scale UASB conditions, as described in the project of Tolmezzo WWTP (Chapter 2).

Some meaningful data about obtained COD removal in the actual pilot-tests were depicted in fig. 5.5. Mean influent COD concentration was 3.25±0.39 g/L, coherent with the results reported in Chapter 3, while effluent COD concentration was 1.51±0.40 g/L: mean COD removal was 53.6%. This result was actually lower than COD removal efficiency expected in Tolmezzo WWTP project (80%), and a further deepening was done, to explain this difference.

As for literature evidences on UASB treatment of P&P WW, limited data were found. A meaningful study, reported in [186], reported UASB treatment of bagasse wash WW, coming from a P&P industry. This WW was characterized from acidic pH (4.5-5.5) and high sCOD concentration (in the range of 2-7 g/L), so it could be somewhat comparable with the actual condensate water stream. In their work, a high COD removal of 80-85%, similar to what reported in Tolmezzo WWTP project, was obtained, coupled with HRT of 20 h and biogas production of 520 L/kg $COD_{removed}$.

Some respirometric tests were planned and executed, to investigate the effective biodegradable fraction of the COD in condensate water: it was supposed that a portion of the total COD was recalcitrant, and so not easily degradable, even in anaerobic environment. This tests were carried out in University laboratories, following the procedure described in [187] using aerobic activated sludge, taken from activated sludge tanks of Tolmezzo WWTP.

A first calibration phase with sodium acetate (NaAc) was performed, to study biomass behaviour in degrading a easily degradable substrate, such as NaAc; in particular, 5 different concentrations of acetate were tested, to calculate calibration curve. The main operations that were performed in this first phase can be summarized as follows:

1. Biomass withdrawal and aeration at ambient T for 1 day, to consume residual substrate and establish endogenous conditions;
2. Thermostatic bath T set at 18 °C and insertion of 0.8 L of sludge in each reactor;
3. DO probe installation, data logging start and sludge oxygenation, until establishing saturation conditions;
4. Aeration maintenance at saturation conditions for 10 min;
5. Aeration stop, after 5 min sodium acetate dosage;
6. After 60 min, test stop.

The second phase, then, consisted in testing the actual substrate (condensate water) with the biomass, using a discontinuous aeration regime, in order to evaluate the capacity of oxygen consumption and re-aeration. The main operations, in this case, were the following:

1. Biomass withdrawal and aeration at ambient T for 1 day, to consume residual substrate and establish endogenous conditions;
2. Thermostatic bath T set at 18 °C and insertion of 0.8 L of sludge in each reactor;

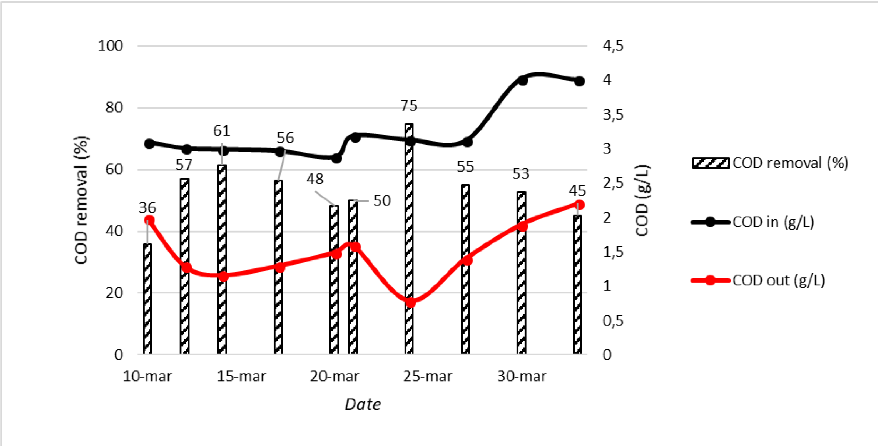

Figure 5.5: Results from UASB tests on condensate P&P WW

3. DO probe installation, data logging start and sludge oxygenation until establishing saturation conditions;

4. Timer start, with a regime of 2 min on-4 min off;

5. After 1 h, substrate dosage, at concentration of 50 mg COD/L biomass;

6. After 24 h, test stop.

Actually, the results from respirometric analysis, differently from what could be expected, revealed the full biodegradability of this matrix, in a percentage proximal to 100%; so, the low COD removal in UASB tests should be explained with the start-up phase of the system, that did not reach fully stable conditions.

In addition, in this phase a deepening campaign was conducted on condensate water tests, to analyse sulphur compounds distribution in condensate water influent and effluent from UASB pilot plant; sulphur, in fact, needs to be accurately monitored, in order to properly dimension H_2S removal equipment, used to protect downstream CHP unit.

Because the historical process data, available at plant level, were mainly related to inorganic sulphur (sulphide, sulphite, sulphate), also organic sulphur was evaluated, and, consequently, total sulphur was obtained, as sum of organic and inorganic sulphur fractions. In the following, the mean data from these analysis were reported and discussed.

From the results on influent fractioning, reported in fig. 5.6, it could be seen that actually organic sulphur accounted for more than 50% (53.0 mg/L, out of 98.1 mg/L) of total sulphur in condensate water. Moreover, sulphite was moderately present in the influent (7.9 mg/L), while sulphate was the main inorganic form (37.2 mg/L); however, it must be remembered that sulphite quickly oxidises to sulphate, so its concentration was probably underestimated. No sulphide was found in the influent.

As for sulphur fractioning in UASB effluent, shown in fig. 5.7, it was evident that sulphur was present mainly in its inorganic forms; this result could be explained with organic compounds degradation, that happened in anaerobic environment. Mean residual organic sulphur concentration was evaluated as 4.5 mg/L (91% reduction). Moreover, a higher sulphite concentration (23.3 mg/L) was detected, if compared to the influent, indicating some reduction of the other sulphur forms. However, the most concentrated compound was again sulphate (41.6 mg/L); total sulphur in the effluent was 70.4 mg/L, with a mean 28% sulphur reduction (that was probably transferred to the gaseous phase, as H_2S).

Finally, to complete this research phase, a punctual detailed analysis campaign was conducted, to measure some meaningful parameters, both in the influent and in the effluent of pilot-UASB unit; the analysed parameters were greases and oils, TKN, formic acid, acetic acid, propionic acid, butanoic acid, iso-butanoic acid, valeric acid, iso-valeric acid. The results from this campaign were shown in tab. 5.1. As could be expected, TKN concentration was very low both in the influent and in the effluent, given the low nutrient concentration present in this matrix; this result, however, was already highlighted in Chapter 3.

A high concentration of acetic acid was measured in the influent, and it increased even more in the effluent: this indicated that an intense acidification occurred, even if it was not completed (as highlighted also from obtained COD removal), to fully degrade the wastewater and complete the AD process. Finally, little concentration of formic acid appeared in the effluent, that probably came from degradation of more complex organic acids (such as propionic and iso-butanoic acid).

5.4 OFMSW leachate tests

OFMSW was obtained from Tolmezzo canteen (differently from characterization phase, where organic waste coming from Udine university canteen waste was

Table 5.1: Pilot-UASB: influent and effluent analysis on condensate water

Parameter	Influent conc (mg/L)	Effluent conc (mg/L)
Greases and oils	14	13
TKN	2.5	5.0
Formic acid	<1	28
Acetic acid	1,440	2,450
Propionic acid	28	<1
Butanoic acid	2	<1
Iso-butanoic acid	18	<1
Valeric acid	<1	<1
Iso-valeric acid	1	<1

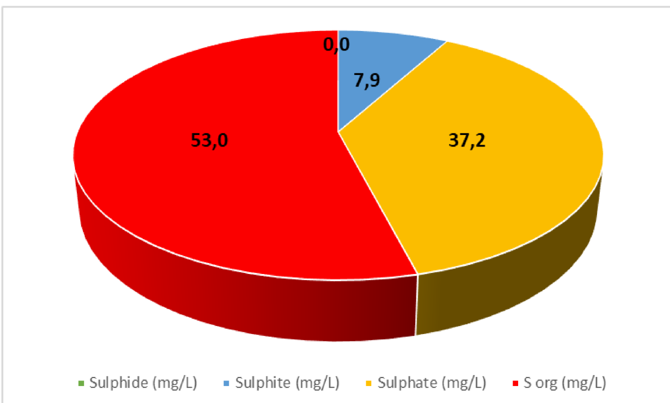

Figure 5.6: Results from sulphur fractioning on condensate water (mean values)

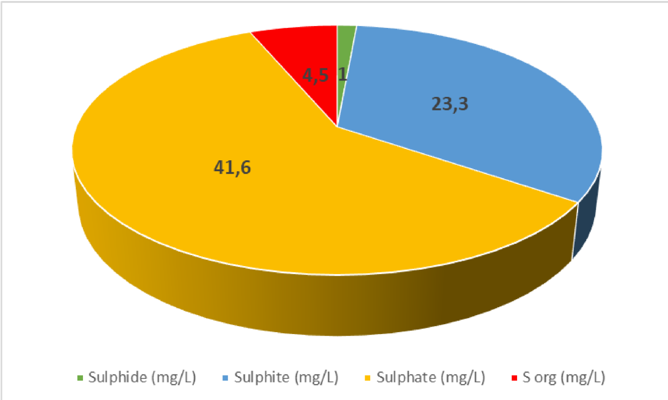

Figure 5.7: Results from sulphur fractioning on anaerobically treated condensate water (mean values)

used) and was manually selected, to remove non-biodegradable materials. It was ground and put in the percolation bed without delay, then tap water was added and leachate was extracted.

In this case, differently from the protocol followed in laboratory tests, contact time between waste and water was shorter, because of the limited amount of available waste (20 kg for each extraction) and the contemporary necessity of producing high volumes of leachate, for continuously feeding UASB pilot-plant. Water-to-waste ratio, in fact, was as high as 10:1, so 200 L of leachate were produced for every waste cycle. Obviously, obtained COD values in leachate were consistently lower than that reported in Chapter 3, because of the shorter contact time: this fact influenced also the continuous tests results. It could be pointed out that waste percolation does not appear to be particularly practical and effective to apply at pilot (and consequently real) scale, so different liquid-solid separation methods (such as mechanical pressing, through screw press) should be employed, to obtain better results. In fact, huge amounts of clean water should be added, and this practice is not sustainable at all.

Despite these general considerations, some results from pilot tests on OFMSW leachate can be reported. A higher flowrate was used in this case (if compared to whey tests), due to the substantial biodegradability of this matrix: Q was as high 259 L/day, corresponding to a mean HRT of 6.0 h. Up-flow velocity, in this

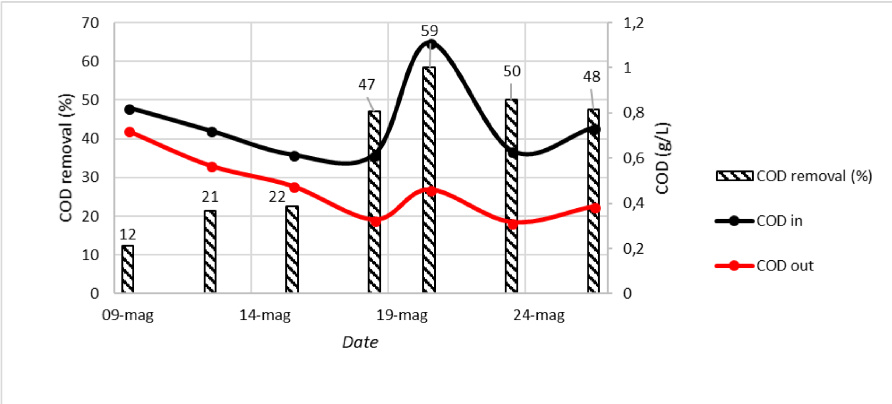

Figure 5.8: Results from UASB tests on OFMSW leachate

conditions, was calculated as 0.40 m/h. Mean obtained methane production in biogas line was 0.13 Nm^3 CH_4/kg $COD_{removed}$. Also in this case, as reported in the previous pilot-tests, only some puntual data of biogas production were available.

The results regarding COD removal were reported in fig. 5.8: mean influent COD was 0.75±0.16 g/L, while mean effluent COD was 0.47±0.13 g/L, corresponding to a mean COD abatement of 37.0%. Actually, as expected, a better COD removal (58%) was observed in correspondence to the peak in influent COD (1.11 g/L); this suggests that lower water-to-waste ratio should be used, to increase both process efficiency and biogas production.

Moreover, the influence of solid-liquid separation process on residual solid fraction characteristics should be investigated, to evaluate the eventual impact on downstream composting process, and in order to study the actual feasibility of upgrading this system.

In literature, as previously mentioned in Chapter 3, an interesting method for treating OFMSW in a mixed solid-liquid reactor was proposed in [96]: a Leach Bed Reactor was used as first-stage, followed by a UASB. Different flow-rates and recycle ratios were tested, and the best operating condition was reported to be a low recycle ratio from UASB to LBR, that lead, in fact, to a 81.8% VS reduction.

Table 5.2: Results from odour analysis on selected matrices samples

Matrix	Influent (ou/m^3)	Effluent (ou/m^3)	Biogas (ou/m^3)
CW	59	500±170	5,533±1,893
Condensate water	4,900	23,000±5,354	84,333±43,438
OFMSW leachate	4,200	11,700±5,749	93,000±21,276

5.5 Odour test results

Odour tests were conducted to analyse the relative impact of the anaerobic treatment of each matrix (CW, condensate water, OFMSW leachate) in smell nuisance contribution; to this purpose, air samples were withdrawn from influent tank, biogas headspace and effluent surnatant during each different set of test. The samples were withdrawn and analysed in an external dedicated laboratory, that operated in standardized conditions, in compliance with international standard UNI EN 13725:2004.

Odour measurements were performed through dynamic olfactometry technique: the dilutions number, necessary to reduce odour concentration to the detection threshold, was expressed as the odour concentration index, and its measurement units were odourimetric units per meter cube of air (ou_E/m^3).

The results from these tests were reported in tab. 5.2. It could be observed that low odour concentration was registered in CW tests, both in storage tank, in effluent and biogas line, probably due to its high dilution, that was actually used for the tests; thus, higher odour concentration is expected to appear, when using lower water-whey ratios. On the other hand, significant odour concentration (up to 93,000 ou/m^3) was present both in condensate water and OFMSW leachate tests; furthermore, odour concentration was very similar between these two matrices, both in the influent tank (4,200-4,900 ou/m^3) and in biogas (84,300-93,000 ou/m^3).

In full-scale operations, an efficient odour removal should be planned, to prevent any smell nuisance to workers, as well as to people living near the plant; actually, in Tolmezzo WWTP high-efficiency chemical scrubbers are operating, able to clean all exhaust air coming from the covered WWTP basins.

5.6 Conclusions

In this chapter, the main findings from pilot-scale continuous UASB tests, executed on selected substrates (CW, condensate P& P WW, OFMSW leachate) were presented. In particular, it was highlighted that:

- CW could be successfully treated, with high removal efficiency, using UASB technology; however, a high dilution should be used, in particular in start-up phase, to avoid operational problems. When the system reaches stable conditions, COD concentration, and consequently OLR, can be increased, so higher methane yields can be obtained, as well. This is coherent with other literature studies, that showed the feasibility of CW treatment in UASB reactors, even using high OLR;

- OFMSW leachate was easily obtainable through waste percolation at laboratory-scale, while at pilot-scale this process appeared less competitive, if compared to mechanical separation (for example, through screw pressing). High volumes for the leaching bed, in fact, were required, together with elevated contact times, not compatible with operational times and amount of available organic waste in the analysed WWTP. Thus, low influent COD was observed in the leachate, that consequently led to a low COD removal in UASB column;

- Condensate water, even if substantially biodegradable, as highlighted from respirometric tests, was digested with a moderate COD removal; nonetheless, once stable conditions are achieved for optimum operation of granular sludge, anaerobic pre-treatment of this matrix appears advantageous, because of its high T and COD concentration, as well as in order to reduce the organic load to activated sludge line in Tolmezzo WWTP.

The work will be completed in Chapter 6, where some energetic data will be discussed, given the results from the previous chapters, also considering, if available, electric and thermal energy consumption of real plants. In particular, local dairies and breweries will be considered, together with an in depth-analysis of the actual production of OFMSW in the analysed territory.

Chapter 6

Energetic considerations

In this chapter, some energetic considerations will be made, in particular on CW, where a high potential for energy and material recovery, as well as real possibility for waste management improvement, is present. In the first section, energy recovery will be analysed, and some energetic data from local dairies will be discussed, in light of the obtained results, while in the successive section material recovery (as proteins) will be described, as an outstanding process for obtaining valuable compounds, that have both an economic and environmental value.

Energy recovery from OFMSW liquid fraction will be quantified as well, by considering the actual amounts of produced waste in the mountain area of Friuli-Venezia Giulia region, and some suggestions to improve its valorisation will be drawn.

Finally, energy consumption from a selected brewery in the analysed territory will be analysed, together with an estimation of possible energy recovery from the different organic wastes (BSG, yeast, whirlpool residue, end-of-fermentation beer), compared to actual electricity and heat consumption.

6.1 Energy recovery from CW

6.1.1 General considerations

The dairy industry plays an economically important role in the agricultural sector; CW, that represents approximately 90% of the employed milk (from a massive point of view), is challenging to dispose, in particular for Small to

Medium Enterprises (SMEs), because typically they do not possess the economic resources required for a proper treatment and valorisation [86]. In fact, these companies usually prefer to give away this residue for farm animal feeding (this is actually done also by the analysed dairies, located in Friuli-Venezia Giulia mountain area), and, sometimes, untreated CW is directly discharged into the municipal sewage system, causing serious environmental hazards, as well as significant problems to municipal WWTPs [81].

Anaerobic digestion (AD) can be a triple action process for CW treatment: pollution discharge reduction, energy obtainment, and nutrient recovery [87]. The successful application of AD to CW depends on the physicochemical composition of CW, in terms of organic matter, reduced alkalinity, rapid acidification tendency, as well as on the inoculum source (that needs to provide high buffer capacity) and reactor configuration [188].

In fact, inhibition by acidification is a common problem encountered during AD of acidic substrates, such as CW. This was actually experienced also in this work, in particular in BMP tests, executed at I/S=2 (Chapter 4), where methane production stopped just after a few digestion days. However, by increasing I/S ratios, high CH_4 production was obtained from selected CW samples. For SMEs, in literature it was suggested to use low-cost tubular digesters, that improve process stability, through separation of acidogenic and methanogenic phases; this solution can be particularly interesting for little facilities, that are not typically able to sustain high investment costs [189].

AD is known for its effect on organic matter stabilisation and removal; however, tipically most of the nutrients remain in the digestate, that is characterized by N/P ratios between 2 and 4 [190]. Although this digestate has good fertilizing properties, its direct application to crops has disadvantages, such as ammonium emissions during irrigation [191] and introduction of pathogens to the fields [192].

To solve this issue, in recent years practical solutions have been proposed, to recover nutrients from the digestate, such as struvite (magnesium ammonium phosphate hexahydrate, $MgNH_4PO_4 \cdot 6H_2O$) [193]. Struvite is formed as crystals (fig. 6.1), that naturally precipitate when the molar ratio $Mg:NH_4:PO_4$ is above 1:1:1 [194], and is characterized by a lower water solubility, in comparison with commercial fertilisers, improving its yield and inhibiting the uncontrolled dispersion of nutrients in the environment [195]. It should be observed that only 20% of the N consumed by cows is present in milk and meat, while the other 80% is disposed of as manure and urine; so, an inappropriate digestate application allows NH_3 and NO_x emissions.

Struvite, instead, is considered a high quality fertilizer, a fire retardant, and an absorbent for removing pollutants from the soil [197]. Transformation of

Figure 6.1: Struvite granules, recovered from liquid hog manure [198]

digestate nutrients into struvite is an environmentally friendly and sustainable method, that can remove residual pollutants, and yield profits for waste treatment in rural and mountain areas.

As for UASB process, that was the main focus of this research, the high concentration of phosphates found in raw cheese whey (Chapter 3), and the limited nutrients removal, that was typically observed in UASB processes, boost for nutrients recovery from the effluent, even if it must be underlined that Mg content in dairy effluents is not sufficient, and must be integrated. In general, phosphate content (high concentrations of PO_4^{3-}, > 500 mg/L, were found in the analysed whey) determines the maximum amount of struvite that can be obtained after precipitation [86].

It is therefore possible to recover both energy (through biogas) and nutrients (through struvite precipitation) from CW. As for general technical considerations, it must be considered that CW production varies during the year, because of climatological conditions, and CW storage (that can be performed in tanks, such as the one shown in fig. 6.2) could be a solution, to compensate for the lack of substrate during the dry season. CW storage involves a decrease in organic matter content and pH, but it has been shown that this does not significantly influence methane production [196].

Given the fact that all the analysed dairies were SMEs, however, it appears advantageous to choose simple technologies for valorising this substrate, rather than UASB processes, that could be tricky to design and operate: choosing

Figure 6.2: CW storage tank [199]

a simple configuration, such as low-maintenance tubular digesters, each dairy could operate its own reactor, and the obtained biogas could be used locally, reducing energy consumption.

In addition, a synergistic effect would be obtained if AD technology beneficiaries were the milk producing farms, that furnish raw milk to the dairy companies, because cattle farms have access to manure, that can be used as inoculum for AD reactors start-up, and CW could be transported by unifying milk collection route from the farms with the transport of whey back from the dairy companies. Payback period for the installation of a simple plastic tubular digester, coupled with struvite precipitation, was calculated as one year in [189], so the feasibility of whey AD was confirmed also at little scale, and a synergism between SMEs and dairy farms should be encouraged, for energy production and nutrients recovery purposes.

6.1.2 Energy recovery potential from selected diaries

Some starting data regarding dairies production can be reported from the analysed plants: a first local dairy reported a raw milk consumption of 60 t/week, together with a CW production equal to 86.5% of raw milk consumption (51.9 t/week), meaning a yield in final products of 13.5%. Considering 5 working days per week, and taking into account whey density (1.03 t/m^3), daily CW volumetric production was calculated as 10.1 m^3/d. A second dairy reported a daily whey production of 11 t/d; again, considering CW density, a volumetric CW production of 10.7 m^3/d was obtained. From these data, it could be seen, indeed, that the amount of produced CW from these two dairies was very similar. Moreover, the yield in final products was consistent with other dairies, located in Friuli-Venezia Giulia, where a yield range of 10.5-13.0% was claimed; generally speaking, the yield of these plants appears to increase if a higher spectrum of products (including, for example, mozzarella and soft cheeses) is made.

Globally, in the analysed territory (Carnia, Val Canale and Canal del Ferro) 5 dairies are present; because a couple of them have higher dimensions than the two reported dairies, total CW production in the area was estimated considering a multiplying factor, that, following some preliminary evaluations, was set at 1.3: consequently, total daily CW production was calculated to be 69.4 m^3/d. As for the transport and disposal costs, that was actually born by the facilities, a standard cost, equal to 90 €/m^3, was considered for the economical evaluations: a global weekly cost of 31,240 €/week was thus obtained.

According to the results of physicochemical characterization, a mean COD concentration of 93.4 g/L (considering a mixture of 50% first CW and 50% second CW) was considered, and the corresponding daily COD production was evaluated as 6,484 kg COD/d. According to BMP tests results, reported in Chapter 4, CW methane potential, at the optimal operating conditions, was 360.4 NL CH_4/kg COD_{added}. Global methane production, considering a complete anaerobic valorisation of the produced whey, was indeed calculated as 2,337 Nm^3/d.

Methane energetic potential is known to be 35.16 MJ/Nm^3: consequently, theoretical energy, available for electricity and heat production (for example through CHP systems), could be estimated as 82,158 MJ/d. In order to make a good estimation of the real electric and thermal energy, effective yields from full-scale CHP systems were considered in the following. Theoretical available energy from CW, if expressed in kW, was as high as 951 kW; a CHP unit system [202], which had a gas consumption of 1,073 kW, was thus considered: the CHP-producing company claimed, from this equipment, an electrical yield of 37.5%, together with a thermal yield of 52.5%. Thus, obtainable electric and thermal energy were calculated, respectively, as 8,558 kWh_{el}/day (corresponding to 357 kW_{el}) and 11,981 kWh_t/day (corresponding to 499 kW_t).

Furthermore, considering a mean yield of 13.5% in final cheese product, globally raw milk consumption in the area could be evaluated as 80.2 m^3/d. Indeed, considering a mean EE consumption of 0.054 kWh/kg milk (obtained from a broader energy analysis, reported in [200]), daily electric energy consumption from local dairies can be estimated as 4,331 kWh_{el}/day. As a consequence, AD from all the produced CW in the area could produce approximately twice the total EE need: excess EE production could be valorised and sold to the gas grid; also, significant incentives are available nowadays for renewable energy production, that could further augment economic income.

As for thermal energy, instead, using a mean specific thermal consumption of 0.410 MJ_t/kg milk [200], corresponding to 0.114 kWh_t/kg milk, total thermal energy consumption from local dairies could be estimated as 9,143 kWh_t/day. Consequently, the heat recoverable from biogas could be sufficient to cover also heat demand from the local dairies.

Finally, to conclude this analysis, it should be pointed out that significant distances occur between local dairies, so the feasibility of adopting diffused AD reactors, localized in each dairy, would probably be the best option, rather then a centralised plant (as was supposed in this paragraph); thus, each plant should consider investment costs for simple AD reactors, together with the availability of specialized personnel, to conduct the plant, as well as the economic income, that could be obtained from EE, in order to choose the best solution to valorise the produced CW. A pay-back time of 3-4 years could be enough low to encourage the diffusion of this technology; however, a further study is required, in collaboration with local dairies, to establish the ideal solution for each dairy, taking into account the specificities, obviously encountered in each process.

6.1.3 Energy consumption in selected dairies

An in depth analysis was made, to analyse the total energetic consumption in local dairies: electric energy, thermal energy and water consumption were considered to this purpose. Selected dairies were analysed, and some meaningful data were reported in tab. 6.1; for thermal calculations, the boiler, used for heat production, and fuelled with natural gas, was assumed to have an efficiency of 85%, while the calorific value of natural gas was estimated as 10.77 kWh/Sm^3 [201].

It could be observed from tab. 6.1 that Dairy 2 had a raw milk consumption more than double than Dairy 1. Specific water and energy (both electric and thermal) consumption appeared to be higher in Dairy 2, if compared to Dairy 1. In addition, cheese yield from selected dairies was coherent with typical reported literature values.

Table 6.1: Water and energy consumption in selected dairies

Parameter	Dairy 1	Dairy 2
Raw milk consumption (t/year)	1,943	5,136
Total cheese production (t/year)	209.8	667.0
Cheese yield (%)	10.8	13.0
CW production (t/year)	1,733	4,720
EE spec consump (kWh/kg milk)	0.027	0.044
Heat spec consump (MJ/kg milk)	0.317	0.557
Water spec consump (L/kg milk)	1.02	3.03

In [203], specific energy consumption from a traditional cheese factory, that produced 1,620 kg mozzarella/day, was reported: they claimed a yield of 0.123 kg mozzarella/L milk (coherent with the values shown in tab. 6.1), an electricity consumption of 0.025 kWh/L milk, a heat consumption of 0.014 MJ/L milk and a water consumption of 2.23 L/L milk. Considering 5 working days per week, a total production of 421.2 t/year was estimated, that was similar to cheese production from selected dairies. It could be interesting to observe that electricity consumption in [203] was practically the same as Dairy 1, while heat consumption was significantly lower (by one order of magnitude), if compared to the analysed dairies. Finally, water consumption was well comparable to that of the actual plants.

A meaningful study, reported in [204], analysed energy mix profile and energy efficiency of the Brazilian dairy industry. A broad range of dairies was taken into account, and some benchmarks, as for EE and heat consumption, were indicated. In particular, as for electricity, a benchmark specific cost of 0.1417 R$/L milk (corresponding to 0.0318 €/L milk) was reported, while, as for thermal energy, a benchmark specific cost of 0.0120 R$/L milk (corresponding to 0.0027 €/L milk) was claimed. A comparison with the actual energy costs for local dairies could be made: as for EE, a standard cost of 0.20 €/kWh was considered, and, starting from the data reported in tab. 6.1, a specific cost of 0.0054 €/L milk and 0.0088 €/L milk was obtained, respectively, for Dairy 1 and Dairy 2. Thus, it could be seen that this cost was significantly lower than that reported in [204], and could be explained with a higher general efficiency of the process.

As for thermal energy, instead, a mean gas cost of 0.25 €/Sm^3 [205] was considered for the successive calculations; a specific cost of 0.0258 €/L milk and

0.0042 €/L milk, respectively, was obtained for Dairy 1 and Dairy 2. It could be seen that actual costs were generally higher than benchmark value. It could be concluded, indeed, that some efficiency increase can be still achieved, as for thermal energy, in the local dairies.

However, it should be taken into account that total energy consumption data, furnished by processing plants, could be referred only to processing equipment, but also, in some cases, to complementary auxiliary units (such as refrigerators), so they were not standardized at all.

6.2 Protein recovery from CW

Over the past few decades, there had been an increasing interest in CW utilisation for the production of highly valuable products, such as whey proteins. Whey processing into these products helps to reduce environmental pollution, and provides the dairy industry with an added economic incentive [206].

Proteins, that are present in whey, are a mixture of globular proteins, and have excellent nutritional properties. Generally, there exist two kinds of recovered proteins, namely whey protein and casein. The latter is a phosphoprotein and is used as a food additive, a binder and as source of carbohydrates, amino acids, calcium and phosphorous. Whey protein, instead, is a great source of amino acids, and is available in various forms; its quantity in CW typically ranges from 6 to 10 g/L [207]. Whey protein chemically consists mainly of β-lactoglobulin (β-Lg) and α-lactalbumin (α-La).

Recovered whey proteins can be made commercially available in various forms, such as highlighted in the general scheme of fig. 6.3:

- Whey Protein Concentrate (WPC) (fig. 6.4): processed form of whey protein, which has the lowest level of fats and cholesterol, and is characterized by a high level of bioactive compounds. Its protein content is in the range of 65-70% ([208]), and it contains, as well, carbohydrates, in the form of lactose [206];

- Whey Protein Isolate (WPI): WPI is a whey protein that has been further processed, to remove fats and lactose. It has lower quantities of bioactive compounds, but a higher protein content (>90%) [206];

- Whey Partial Hydrolisate (WPH): WPH are pre-digested and partially hydrolysed whey proteins, with an easier metabolism. Protein content is 70-80% [206];

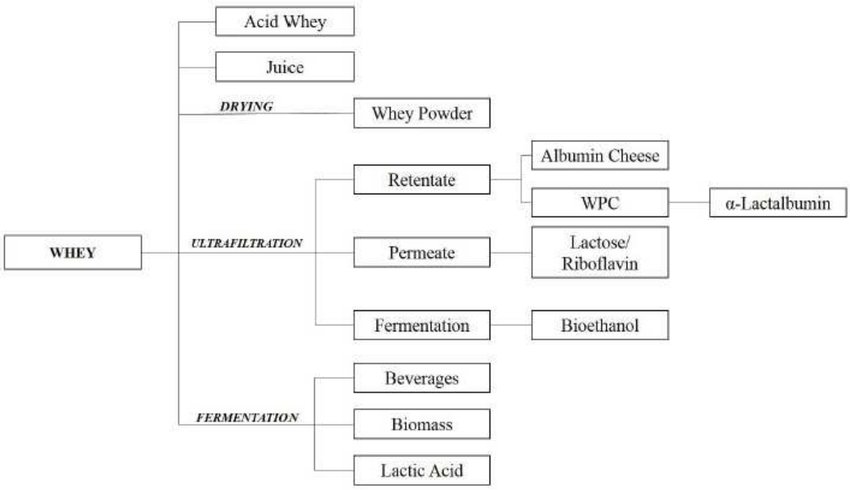

Figure 6.3: Added value products that can be obtained from whey [207]

- Native Whey: it is extracted from skimmed milk and can be produced as concentrate or isolate, even if it has limited uses [206].

Whey protein separation process essentially consists of three stages [206]:

1. Pre-treatment, aimed at avoiding problems in the following phases;

2. Separation: several methods have been used to separate CW into its various components. These techniques are mainly based on membrane separation processes, with ultrafiltration being the highest contributor. The major operating problem, commonly encountered in daily operations, is membranes fouling, that leads to a reduction in flux and efficiency in whey protein separation;

3. Drying, which as the purpose to reduce transportation costs and increase the product shelf life. The most common drying method is spray drying; optimization of heat application to separated whey proteins is crucial, because protein denaturation should be prevented, in order to guarantee shelf life of the final product [206].

Given the outstanding interest of this process, in the following sections a brief description of the three phases for whey protein separation will be made; as

Figure 6.4: Whey protein concentrate [209]

for general considerations, these processes require significantly higher investment costs, if compared to energy recovery, as described in the previous paragraphs. Indeed, given also the strong technical complexity, SMEs cannot typically afford to implement resource recovery plants; nonetheless, in the future this solution appears to be very competitive, because of the broad spectrum of high-value products that could be obtained. Successive studies are required, anyway, to evaluate the possible application of this technology in a particular territory, such as the mountain area of Friuli-Venezia Giulia region.

6.2.1 Pre-treatment

As already stated, CW pre-treatment is needed, in order to induce selective changes, to limit the negative effects of fouling in the successive membrane separation stage. Nowadays, the most popular pre-treatment methods, used in the dairy industry, are [206]:

- Chemical pre-treatment: it involves the addition of chemicals to whey, with the aim of removing the components that contribute to membrane fouling [206];

- Thermal pre-treatment: controlled application of heat, to improve the shelf life and stability of protein solutions and beverages. It is typically used to reduce proteins aggregation, that may cause the solution to become turbid [206];
- Ultrasound (US), that helps to breakdown protein aggregates and improve the heat stability. Often US pre-treatment is combined with heat pre-treatment, giving higher efficacy. In membrane systems, US application can increase flux, as well as improving cleaning efficiency [206];
- Turbulence promoters, that enhance turbulence and shear near the membrane surface [206].

Despite of the chosen pre-treatment, membranes often need to be cleaned, to remove the detrimental deposits and restore the initial permeation properties. Conventionally, cleaning is performed using an alkali cleaning step, followed by an acid cleaning step. Also, non-conventional cleaning methods have been developed, such as US cleaning, use of saline solutions, use of electric fields [206].

6.2.2 Membrane filtration

In ultrafiltration (fig. 6.5), hydrostatic pressure forces the liquid against a semi-permeable membrane, which leads to the retention of suspended solids and solutes having high Molecular Weights (MW); water and lower MW solids, instead, can pass through the membrane. Ultrafiltration offers a unique method for whey protein recovery in their native form. The typical MW cut-off for this process is 10 kDa; the process is normally operated at T<55 °C and inlet pressure of 300 kPa, while membrane pore size is in the range of 200-250 nm. Ultrafiltration can increase protein content of whey up to 85% [206].

An evolution of the single-step process consists of the cascaded ultrafiltration, where either the retentate or the permeate from one membrane stage is transferred to the next or previous stage as feed; there can be also some recycling. This configuration can lead to an additional enrichment of whey proteins. Moreover, it has been showed in literature that, through cascaded ultlrafiltration, separation efficiency can be significantly enhanced, if compared to single stage operations [206].

Another process improvement can be bio-catalysts addition: it has been demonstrated that large sized proteins have less influence on membrane fouling and are also easier to clean, if compared to small-sized proteins. Bio-catalysts are enzymes capable of cross-linking proteins, resulting in the formation of high

Figure 6.5: Ultrafiltration module for whey processing [212]

molecular polymers, that can be easily retained on the exterior surface of the membrane [210].

Summarizing, the main advantages of membrane separation process are the following [206]:

- It is a non-thermal and environmentally friendly technology;
- Various methods can be used to increase membrane selectivity; also construction materials can be chosen in order to give a higher affinity for certain proteins, according to the specific purpose;
- Membrane configuration is suitable for easy industrial application, due to the compact design and low required maintenance;
- Specialised knowledge base is not necessary, to operate membrane modules.

Undoubtedly, as already specified, the major process limitation is fouling, that is caused by a combination of different phenomena, such as concentration polarisation, pore blocking or cake formation; fouling limits mass transfer, because of deposits formation. Reversible fouling happens when the deposits can be easily removed, by rinsing water through the ultrafiltration membrane, while, on the other hand, irreversible fouling refers to a layer that can only be removed using chemical cleaning [206].

Some new methods have been recently developed, to enhance protein recovery potential; among them, the following are worth mentioning [206]:

- Membrane distillation, that is a thermally driven process. Water vapour is allowed to pass through the membrane, and the flux is driven by the vapour pressure gradient;
- Anion exchange membranes, where the separation is driven by electrostatic interactions between the surface charges on biomolecules and clusters of charged groups on membranes;
- Precipitation, achieved by heat introduction or specific chemicals addition. Heat introduction leads to aggregates formation, that settle down and can be easily removed. However, the selection of chemicals should be very accurate, because they may lead to chemical properties alteration.

6.2.3 Drying

Once the proteins have been isolated, they need to be dried, to eliminate moisture and respect commercial requirements for final products. This process also increases physical and microbiological stability of whey proteins, allowing for a reduction of transport and storage costs. The main processes, industrially applied, are spray drying and freeze drying [206].

Spray drying (fig. 6.6) is an effective process, because it removes water at the lowest T and in the shortest time. It is based on the generation of very fine droplets, using a nozzle or a rotary atomizer, into a hot dry air system, typically at T of 180-220 °C [211]. The concentrated whey protein enters the spray dryer chamber and exits, as powder, at the bottom; several cyclones are placed outside the dryer, in order to remove dry products from humid air. Finally, drying and cooling occur in a fluidized bed, where the powder from the end of the chamber and from the cyclones mix together.

The advantages of this method include rapid drying, large throughput and continuity of operations, but, on the other hand, high operation costs are encountered, together with some unavoidable protein denaturation. Recent advancements in this technology include the replacement of conventional nozzles with ultrasonic nozzles, that are more precise, reliable and give controlled distribution [206].

Freeze drying, instead, is used to produce high-quality dried products, which are sensitive to heat [214], and is based on the use of T below liquid freezing point. The process is operated under vacuum; the component to be dried is

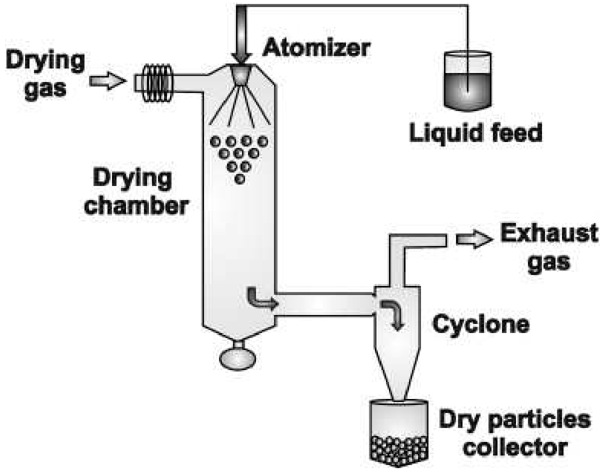

Figure 6.6: Process scheme of CW spray drying [213]

first frozen, and then exposed to heat under vacuum, with causes sublimation of the frozen liquid. When choosing this process, most deterioration reactions are retarded to a great extent; in fact, freeze drying is considered to be the best method for the production of high quality dried products. However, freeze drying is expensive from an energetic point of view, and the throughputs are limited, if compared to spray drying [206].

An evolution of these processes is spray-freeze-drying (a combination of the two methods), where the solution is sprayed into a cold medium, and the resultant frozen particles are then freeze-dried, by putting them in contact with a cold, dry gas stream in a fluidized bed. This process enables a much faster drying than it is usually possible by conventional freeze-drying [215].

6.3 Energy recovery from OFMSW liquid fraction

AD process for OFMSW treatment is nowadays a mature technology, with over 560 plants for power generation reported worldwide, having a combined capacity of over 7.3 TWh/year [216]. The use of energy derived from biogas, instead of fossil fuels, helps to reduce CO_2 emissions at levels of 200-300 kg CO_2/t waste;

moreover, the use of digestate, in place of mineral fertilizers, provides a further reduction in CO_2 emissions by 30-40 kg CO_2/t waste [217].

As for OFMSW separation into a liquid and a solid fraction, a meaningful example is that of Treviso municipality, where a strong collaboration exists between the waste managing company (Contarina) and IWS local authority (ATS): high purity organic waste is collected, and it is mechanically pressed, to obtain a highly biodegradable liquid fraction, that is then used in the local WWTP in co-digestion with sewage sludge, significantly increasing biogas yields.

The successive calculations for the actual quantities of available organic waste in the analysed area were made considering the municipalities of the Mountain Communities of Carnia and Gemonese, Val Canale, Canal del Ferro: globally, 43 municipalities were present, for a total population of 67,820 inhabitants (year 2017). Organic waste production, corresponding to the European Waste Code (EWC) 200208, globally was, in 2017, as high as 4,141.5 t/year, and the specific OFMSW production was thus evaluated as 67.8 kg OFMSW/inhab year.

As already mentioned, in [105], a simplified mass balance for solid-liquid separation of OFMSW was reported: the liquid fraction corresponded to 30% (on mass basis) of the OFMSW total mass. So, considering the actual amount of waste produced in the analysed territory, it could be possible to extract 1,242.5 t/year of high-loaded liquid fraction, while the residual solid fraction, to be composted, would be 2,899 t/year. Assuming BMP of OFMSW liquid fraction as equal to that reported in [105] (0.49 m^3 CH_4/kg VS, with 118 g VS/L and a density of 1,020 kg/m^3), the volume of CH_4 that could be obtained yearly through AD would be 70,431 m^3 CH_4/year; then, considering a mean ambient T of 20 °C, the corresponding normalised daily volume was calculated as 180 Nm^3 CH_4/day.

Again, considering methane energetic potential (35.16 MJ/Nm^3), theoretical energy, available for AD processes, could be estimated as 6,329 MJ/day. Considering a micro-CHP unit of 68.4 kW, having electric and thermal yields respectively equal to 32.2% and 73.1% [202], real electric and thermal energy production would be 566 kWh/day and 1,285 kWh/day.

If compared to the obtainable CH_4 and energy yields that could be obtained from CW (reported in section 6.2), a difference of one order of magnitude comes out: actually, given the low population density of this territory, an implementation of an AD plant for liquid fraction, coming from OFMSW, appears competitive only in the case of using an existing WWTP (for example Udine WWTP) for a co-digestion process.

In this case, co-digestion of OFMSW liquid fraction and sewage sludge would appear significantly advantageous. Sewage sludge characteristics play

Table 6.2: Energy consumption in a selected brewery

Year	Beer (hL)	EE (MJ/hL)	TE (MJ/hL)	Energy cost (€/year)
2016	2,940	136.4	98.9	24,581
2017	3,190	130.6	112.2	25,969
2018*	2,700	148.9	124.9	25,005

*: January-July

a crucial role in this process: N content in secondary sludge can alleviate the possible lack of nutrients in OFMSW, while primary sludge (rich in lipids) can increase methane production in AD process [218]. This solution, applied to untreated OFMSW (instead of a pre-separated liquid fraction) was already deeply investigated in [179]; nonetheless, a specific focus on co-digestion of press water and sewage sludge could be a suggestion for a possible future work.

6.4 Energy recovery from brewery waste

There are various factors that influence energy consumption pattern in a particular brewery, such as local climate conditions, production technology, product mix, use of different bottling technologies, capacity utilization [219]. Moreover, even if similar technologies are applied for brewing in different breweries, even small differences can influence the energy management to a large extent [220].

Energy and water consumption from a local brewery was analysed in the following; to this purpose, energy costs were evaluated considering a standard electricity cost of 0.20 €/kWh and a conversion coefficient (from m^3 to Sm^3) of 1.0034 for natural gas, while calorific value of natural gas was approximated to 10.77 kWh/Sm^3 and boiler thermal efficiency for heat production was taken as 0.85. The main results from energetic analysis were reported in tab. 6.2, and highlight a moderate increase of energy cost through the years, due to an increase in the amount of produced beer. Limited variations in specific electricity (in the range of 130.6-148.9 MJ/hL) and thermal consumption (in the range of 98.9-124.9 MJ/hL) arise from one year to another.

In [219] an energetic analysis was carried out on a Latvian brewery (having a production of 15,000-17,000 hL/year, so significantly higher than the reported brewery): in particular, a specific thermal consumption of 220-230 MJ/hL was claimed, that was significantly higher than the actual brewery consumption. As for electricity, they underlined a specific EE consumption of 82-92 MJ/hL, that, instead, was lower, if compared to the EE need of the analysed plant.

The results from BMP tests (Chapter 4) were used to estimate total methane yield, that could be obtained by anaerobically digesting all the brewery waste. Yearly production of each waste was considered, and the contribution of biogas to electric and thermal energy consumption was evaluated, by considering an electric yield of 35% and a thermal yields of 55%. The results were summarized in tab. 6.3.

It could be noticed, as highlighted also from literature references, that BSG (trub) and spent yeast were the main residues, from a volumetric and also a massive point of view; moreover, a higher methane production could probably be obtained by spent beer and also whirlpool residue, when working in co-digestion process, or optimizing operating variables, in order to enhance biogas yields. As an example, the addition of biochar (as shown in Chapter 4) would increase in a significant manner the obtained values.

From this basic results, however, it could be estimated that AD from all the brewery wastes could produce up to 18.4% of the total electric need and 27.7% of the total thermal requirement; if biochar could be added, as for spent yeast and whirlpool residue, these percentages would increase up to 21.4%, as for EE, and 32.3%, as for TE. It should be underlined, finally, that the interaction between these matrices in a co-digestion process needs to be further investigated, in order to evaluate eventual synergistic effects (for example between spent yeast and AD sludge), as well as to prevent any possible operating problem.

Finally, as underlined for local dairies, choosing simple digesters, with low moving parts and sustainable investment cost, managed by the personnel employed in the brewery, could be an interesting solution, to increase sustainability, reduce environmental impact, lower energy costs and shift to a circular economy perspective.

6.5 Conclusions

In this chapter, in light of the results of the whole research work, some final considerations on energy and material recovery were made on the substrates where the highest potential for improvement in waste valorisation strategy was found: CW, OFMSW and brewery waste.

In particular, it could be remarked that:

- CW is the substrate whose valorisation appears most feasible and competitive: one possible solution includes a centralized AD plant, that receives

Table 6.3: Energy analysis from brewery waste

Substrate	V (m^3/year)	Mass (kg VS/year)	BMP (NL CH_4/kg VS)	EE (kWh/year)	Therm (kWh/year)
Trub	100	6,990	356	9,911	15,574
Yeast	36	5,027	487	9,744	15,311
Whirl	12	756	290	874	1,373
End beer	12	428	126	214	337
Total	**160**	**13,201**	-	**20,743**	**32,596**

all the whey generated by the local dairies, able to produce electric energy (for injection into the grid) and heat (to sustain the process and, eventually, for district heating). As previously highlighted, high I/S ratio should be adopted, to obtain high methane yields and prevent inhibition by VFA accumulation. However, given the long distance between the analysed plants, a decentralised solution, with small distributed AD reactors, appears more feasible: simple reactor configuration should be privileged, to allow easy operation and low investment cost. This solution could provide most of the electricity and heat needed by the plant, as was shown in 6.1.3, together with a short pay-back period and ease of operations. In larger plants, instead, where a higher investment cost is typically sustainable, resource recovery processes, as described in 6.2, could be applied, given the high economic value of proteins;

- OFMSW production in the geographic area is quite low: it could be interesting to evaluate a co-digestion of the liquid fraction, obtained after mechanical pressing, with sewage sludge, that have already been proved to be sustainable, and is actually applied also in some Italian full-scale plants (such as Treviso WWTP). The solid fraction, anyway, needs to be recovered through composting, as it is already done nowadays in most cases;

- Brewery waste is a highly biodegradable substrate, and consists of an heterogeneous mixture of substrates (trub, spent yeast, whirlpool residue, end-of-fermentation beer). A good solution for valorising this substrates could be the implementation of little AD plants, managed by brewery owners, in order to get a significant share of the electric and thermal energy need of the plant. However, in order to increase biogas yields, efficient pre-treatments and/or proper co-digestion mixtures should be further studied and evaluated. As an example, the meaningful increase in biogas yields, that was observed when adding biochar or GAC, should be deepened, to improve the obtainable yields.

Chapter 7

Conclusions

This Ph.D. research was focused on energy and material recovery from high-loaded liquid substrates, present in the mountain area of Friuli-Venezia Giulia region. UASB anaerobic treatment was studied, as a possible target for energy recovery, given the presence of a UASB reactor in Tolmezzo WWTP; this reactor was actually designed for pre-treating condensate P&P WW.

First and second cheese whey (CW), condensate P&P WW, OFMSW leachate, brewery waste (spent grain, yeast, whirlpool, end-of-fermentation beer) and slaughterhouse liquid waste were the selected substrates, due to the presence of specific plants in the area, and given the possibility to valorise this matrices, whose transport, treatment and disposal is actually cumbersome. The aim of the research study was to select the best treatment and valorisation strategy for each substrate, taking into account environmental, energetic and economic aspects. A percolation bed was assembled for the extraction of a soluble liquid fraction from solid organic waste.

It was showed, through laboratory physicochemical characterization, that CW, condensate water, OFMSW leachate, brewery waste and slaughterhouse liquid waste were strongly polluted streams, and it was highlighted that each substrate had peculiar characteristics, that needed to be taken into consideration, when choosing the best valorising strategy. Moreover, BMP tests and continuous-UASB tests demonstrated that it is possible to anaerobically treat all of these matrices, even if CW has a strong acidification tendency, if not properly diluted, so a high I/S ratio has to be adopted, while condensate water is strongly acidic and poor of nutrients, so proper nutrient addition and pH correction should be planned; in addition, slaughterhouse waste is characterized by a slow hydrolysis, that affects final methane yields in BMP tests.

Ultra-sound (US) pre-treatment was tested on CW, in order to evaluate a possible increase in methane yields; a significant increase both in final BMP yield and methane production kinetics was observed, together with the fact that higher applied US energy did not produce a further increase in BMP value. In fact, no difference between longer US-pretreated and untreated whey was seen, indicating a strong non linear behaviour.

A high dilution of CW was used for continuous UASB tests, in order to allow an easy adaptation of granular biomass, and high COD removal was obtained, together with stable operations. A progressive increase in COD (reducing dilution rate), and consequently OLR, can be planned in successive phases, to increase also biogas yield.

OFMSW leachate was shown to be highly biodegradable, but it was tricky to reproduce at pilot scale the laboratory tests, because of the numerous operating conditions that influenced leachate characteristics, as well as for the necessity of producing consistent volumes of leachate. In fact, low COD concentration was obtained at pilot scale, lowering also obtainable efficiency and biogas production of pilot-UASB system.

Finally, brewery waste gave good methane yields, as for spent grain and yeast, while lower methane generation potential was obtained from whirlpool residue and end-of-fermentation beer. The addition of low amounts of biochar and granular activated carbon significantly enhanced methane production from spent yeast and whirlpool residue, so a further deepening is wortwhile, in order to evaluate biomass sources in the area, that could be used to produce biochar, improving AD process, with environmental, economic and technical improvement. Synergistic effects and proper co-digestion mixtures should be evaluated, to study the actual feasibility of full-scale AD systems, localized at brewery level.

A simplified kinetic analysis was carried out on BMP test results, to obtain some meaningful parameters for AD process, and, where available, energy consumption from full-scale plant was analysed, comparing the results to literature evidences.

As for CW, a strategy to improve current management of this sub-product was suggested: given the high concentration of valuable compounds, the possibility of applying resource recovery, rather then just energetic recovery, should be privileged in large dairies, because of the extra economic income that could be obtained, by selling the recovered products. However, in little facilities, such as the ones studied in this work, due to the high investment costs that are required for protein recovery (through ultrafiltration process), the use of simple anaerobic digesters, with low investment and operating costs, is surely more feasible, for improving the energy balance of processing plants.

Through an energetic analysis it was shown, in fact, that CW AD can provide most of the electricity and heat needed for the dairy, so the localization of AD reactors at dairy scale appears advantageous, given also the significant distance between the processing plants in the analysed area. Due to the fluctuations in whey production, CW storage can be fruitful, to allow digesters to operate continuously. Possible further studies on this matrix include nutrient recovery application (for example in the form of struvite or proteins), as well as pilot-studies for protein recovery, testing real obtainable yields from the selected whey.

As for condensate water, continuous UASB tests underlined a moderate COD abatement, lower than that expected in the technical report of Tolmezzo plant; this was explained with the time needed for granular biomass to adapt to this harsh substrate. Actually, UASB pre-treatment of this stream allows to improve WWTP energetic balance, but, given the peculiar composition of the stream, probably does not significantly affect total process efficiency of the plant, in terms of COD removal, as well as effluent composition. Anaerobic treatment of condensate water, on the other hand, reduces COD load on activated sludge basins, reducing also oxygen demand, thus significantly improving the energy balance, through reduction of oxygen supply.

As for source-sorted organic waste (OFMSW), an extreme variability in leachate characteristics was encountered, due to the high number of variables affecting solid-liquid separation process. It was suggested to study also alternative separation methods, such as mechanical pressing (for example using screw presses), that could significantly enhance COD concentration in the liquid fraction. Liquid-solid separation from the analysed waste could lead to a highly biodegradable liquid fraction, amenable to be co-digested with sewage sludge (for example in an existing municipal WWTP, such as Udine plant). In addition, the influence of waste pre-treatment (eventual grinding and liquid extraction) on waste compostability should be further investigated, for example through specific composting tests, given the fact that solid fraction needs to be properly stabilised, before recovery.

As for slaughterhouse waste, a generally low kinetics in methane production was encountered; this was due to the high presence of proteins and fats in this matrix, revealed in the physicochemical characterization phase. Moreover, sanitary protocols should be followed in its proper treatment and valorisation; the possibility of anaerobically digesting this matrix could be an option, but further research is required, to test, for example, co-digestion with substrates having complementary characteristics.

As for brewery waste, significant differences were highlighted between the analysed substrates. The possibility of anaerobically treating all these matrices, at brewery level, should be further studied: it was shown that a significant share of the electric and thermal energy of the plant could be covered by the obtained biogas. In particular, spent grain and excess yeast actually represent the most valuable streams, to produce biogas, but also whirlpool residue and end-of-fermentation beer should be taken into account. Moreover, synergism effects could probably come out, when co-digesting all the produced brewery waste.

In conclusion, due to a general EU perspective of further increase in renewable energy utilisation, and given the high potential of biogas (and even more biomethane) to further penetrate the market (also in transportation sector), this work can be a good example for improving the collaboration between Universities and research centers, water utilities and manufacturing companies, to study and adopt innovative solution for energy and material recovery, not only from wastewater, but also from solid waste.

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Bibliography

[1] European commission. *Closing the loop-An EU action plan for the circular economy.* Communications from the commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, COM (2015) 614.

[2] C Hageluken, JU Lee-Shin, A Carpentier, C Heron. The EU Circular Economy and Its Relevance to Metal Recycling. *Recycling* **1**(2), 242-253, 2016.

[3] European commission. *The role of waste-to-energy in the circular economy.* Communications from the commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, COM (2017) 34.

[4] DA Vaccari. *Global cycles of Phosphorous and Nitrogen- Resources and Leaks.* Proceedings in Sustainable Water-Energy Centric Communities, Lake Como School, 2016.

[5] Labiotech, https://labiotech.eu/biorefinery-review-europe-biobased, 2018.

[6] IEA. *Biorefining in a future BioEconomy*, 2008.

[7] TM Mata, AA Martins, NS Caetano. Bio-refinery approach for spent coffee grounds valorization. *Bioresource Technology* **247**, 1077-1084, 2018.

[8] F Girotto, A Pivato, R Cossu, GE Nkeng, MC Lavagnolo. The broad spectrum of possibilities for spent coffee grounds valorisation. *Journal of Material Cycles and Waste Management*, 1-7, 2017.

[9] E Ahmetovic, N Ibric, and Z Kravanja. Optimal design for heat-integrated water-using and wastewater treatment networks. *Applied Energy* **135**, 791-808, 2014.

[10] Metcalf and Eddy Inc. *Wastewater engineering: Treatment and reuse* (4^{th} ed.), New York: McGraw-Hill, 2006.

[11] M Molinos-Senante, N Hanley, and R Sala-Garrido. Measuring the CO_2 shadow price for wastewater treatment: a directional distance function approach. *Applied Energy* **144**, 241-249, 2015.

[12] D Panepinto, S Fiore, M Zappone, G Genon, and L Meucci. Evaluation of the energy efficiency of a large wastewater treatment plant in Italy. *Applied Energy* **161**, 404-411, 2016.

[13] H Liu, R Ramnarayanan, and BE Logan. Production of electricity during waste-water treatment using a single chamber microbial fuel cell. *Environmental Science & technology* **38**, 2281-2285, 2004.

[14] G. Olsson. Proceedings in *"Energetic and material recovery issues in modern urban metabolism: strategies and technologies for a sustainable future"* summer school. Lake Como School of Advanced Studies, 21-25 August 2017.

[15] Pike research. *Worldwide power generation capacity from biogas will double by 2022*. Boulder, USA. Research P., 2012. Available at: http://www.businesswire.com/news/home/20121107005284/en/Worldwide-Power-Generation-Capacity-Biogas-Double-2022.

[16] JB Holm-Nielsen, T Al Seadi, and P Oleskowicz-Popiel. The future of anaerobic digestion and biogas utilisation. *Bioresource Technology* **100**(22), 5478–5484, 2009.

[17] C Florio. Biomethane production: biogas upgrading technologies. University Partenope of Naples, https://www.linkedin.com/pulse/produzione-di-biometano-tecnologie-upgrading-del-biogas-ciro-florio, 2018.

[18] Q Sun, H Li, J Yan, L Liu, Z Yu, and X Yu. Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation. *Renewable and Sustainable Energy Reviews* **51**, 521-532, 2015.

[19] C Lau, A Tsolakis, and M Wyszynski. Biogas upgrade to syngas(H_2–CO) via dry and oxidative reforming. *International Journal of Hydrogen Energy* **36**(1), 397–404, 2011.

[20] M Beil and H Hahn. *Biogas upgrading technology overview*. Urban biogas training. Zagreb, Croatia. 2012.

[21] B Anderson and A Montagnier. Analysis of methane losses in a biogas production plant. Jönköping, Sweden, 2002.

[22] Bio.methan, http://bio.methan.at/en/pws, 2018.

[23] LB Allegue and J Hinge. *Biogas and bio-syngas upgrading*. Aarhus, Denmark, 2012.

[24] JD Hullu, JIW Maassen, PAV Meel, S Shazad, and JMP Vaessen. *Comparing different biogas upgrading techniques- final report*. Eindhoven, The Netherlands, 2008.

[25] Y Li and L Yang. Converting Biogas to Transportation Fuels. *Agricultural and Natural Resources*, Ohio University, https://ohioline.osu.edu/factsheet/AEX-653.2, 2014.

[26] L Yang and Y Li. Biogas Cleaning and Upgrading Technologies. *Agricultural and Natural Resources*, Ohio University, https://ohioline.osu.edu/factsheet/AEX-653.1-14, 2014.

[27] L Deng and M-B Hägg. Techno-economic evaluation of biogas upgrading process using CO_2 facilitated transport membrane. *International Journal of Greenhous Gas Control* **4(4)**, 638–646, 2010.

[28] T Patterson, S Esteves, R Dinsdale, and A Guwy. An evaluation of the policy and techno-economic factors affecting the potential for biogas upgrading for transport fuel use in the UK. *Energy Policy* **39(3)**, 1806–1816, 2011.

[29] M Persson, O Jönsson, and A Wellinger. *Biogas upgrading to vehicle fuel standards and grid injection*. In: R Braun, JB Holm-Nielsen, TA Seadi, D Baxter, M Jormanainnen, J Rintala, et al., editors. Task 37: Energy from biogas and Lanfill Gas. Paris, France, 2006.

[30] Air Liquide, https://www.airliquideadvancedseparations.com/our-membranes/biogas.

[31] EBA, *6th Statistical Report of the European Biogas Association*, 2015.

[32] M Mainardis. *UASB anaerobic treatment and OFMSW reutilization: Tolmezzo case potential.* Master Degree Thesis in Engineering for Environment and Energy, 2014.

[33] C Gregoris. *Pilot-scale experimentation and modelling of an activated sludge process supporting pulp&paper wastewater plant management.* Ph.D. Thesis in Chemical and Energetic Technologies- Environment Pollution and Remediation, Udine University, 2011.

[34] Regione Autonoma Fvg. http://www.consiglio.regione.fvg.it/cms/hp/informazioni/0072.html, 2018.

[35] Passavant Impianti Spa and Achille Fadalti Costruzioni Spa. *Executive Project- Design and execution of consortium treatment system up-grade in Tolmezzo municipality.* 2005.

[36] E Marisova, J Milovanovic, Z Jurekova, K Mandalova. *Agro-energy for sustainable agriculture and rural development- Good practices from Slovakia-Serbia bilateral cooperation.* Faculty of Applied Ecology Futura Singidunum, University Belgrade, ISBN: 978-86-86859-53-2, 2016.

[37] L Bonomo. *Wastewater Treatment Manual.* McGraw-Hill, Milan, Italy, 2008.

[38] L Tuccinardi. *Energetic carriers production through organic wastes fermentation.* Ph. D. Thesis in "Environmental Engineering", University La Sapienza, Rome, 2010.

[39] T Abbasi and SA Abbasi. Formation and impact of granules in fostering clean energy production and wastewater treatment in upflow anaerobic sludge blanket (UASB) reactors. *Renewable and Sustainable Energy Reviews* **16**, 1696-1708, 2012.

[40] G Lettinga and LW Hulshoff Pol. UASB-process design for various types of wastewaters. *Water Science and Technology* **24**, 87-107, 1991.

[41] R Petruy and G Lettinga. Digestion of a milk-fat emulsion. *Bioresource Technology* **61**, 144-149, 1997.

[42] EV Ramasami, S Gajalakshmi, R Sanjeevi, MN Jithesh, and SA Abbasi. Feasibility studies on the treatment of dairy wastewaters with upflow anaerobic sludge blanket reactors. *Bioresource Technology* **93**, 209-212, 2004.

[43] Gear Idea, http://gear.searchbran.com/2017/12/23/upflow-anaerobic-sludge-blanket-reactor-pdf, 2017.

[44] S McHugh, M Carton, T Mahony, V O'Flaherty. Methanogenic population structure in a variety of anaerobic bioreactors. *FEMS Microbiology* **219**, 297-304, 2003.

[45] G Lettinga, AFM van Velseo, SW Hobma, and W de Zeeuw. Use of the up-flow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering* **22**, 699-734, 1980.

[46] FI Hai, K Yamamoto, and CH Lee (Eds.). *Membrane Biological Reactors: Theory Modeling Design Management and Applications to Wastewater Reuse.* IWA Publishing, London, 2013.

[47] SS Adav, DJ Lee, KY Show, and JH Tay. Aerobic granular sludge: recent advances. *Biotechnology Advances* **26**(5), 411-423, 2008.

[48] JH Tay, KY Show, DJ Lee, and ZP Zhang. *Anaerobic granulation and granular sludge reactor systems*, in Environmental Anaerobic Technology-Applications and New Development, Imperial College Press, 113-136, 2010.

[49] SM Tauseef, T Abbasi, and SA Abbasi. Energy recovery from wastewaters with high-rate anaerobic digesters. *Renewable and Sustainable Energy Reviews* **19(0)**, 704-741, 2013.

[50] N Mahmoud. High strength sewage treatment in a UASB reactor and an integrated UASB-digester system. *Bioresource Technology* **99(16)**, 7531-7538, 2008.

[51] C Zhao. *Effect of Temperature on Biogas Production in Anaerobic Treatment of Domestic Wastewater UASB System.* Land and Water Resources Engineering, KTH Royal Institute of Technology, Stockholm, Sweden, 2011.

[52] RA Hamza, OT Iorhemen, and JH Tay. Advances in biological systems for the treatment of high-strength wastewater. *Journal of Process Engineering* **10**, 128-142, 2016.

[53] SZ Ahammad, JL Bereslawski, J Dolfing, C Mota, and DW Graham. Anaerobic-aerobic sequencing bioreactors improve energy efficiency for treatment of personal care product industry wastes. *Bioresource Technology* **139(0)**, 73-79, 2013.

[54] A Torkian, A Eqbali, and SJ Hashemian. The effect of organic loading rate on the performance of UASB reactor treating slaughterhouse effluent. *Resources, Conservation and Recycling* **40(1)**, 1–11, 2003.

[55] MA Latif, R Ghufran, ZA Wahid, and A Ahmad. Integrated application of upflow anaerobic sludge blanket reactor for the treatment of wastewaters. *Water Research* **45**, 4683-4699, 2011.

[56] M Blanken, C Verweij, KF Mulder. *Innovation in sanitary systems. Why are novel sanitary systems hardly introduced?*. Sustainable Development in Energy, Water and Environmental Systems. Dubrovnik, October 2017.

[57] MH Gerardi. *Wastewater Bacteria.* Wiley-Interscience, New Jersey, 19-31, 2006.

[58] W Parawira, M Murto, R Zvauya, and B Mattiasson. Comparative performance of a UASB reactor and an anaerobic packed-bed reactor when treating potato waste leachate. *Renewable Energy* **31**, 893-903, 2006.

[59] N Buyukkamaci and A Filibeli,. Volatile fatty acid formation in anaerobic hybrid reactor. *Process Biochemistry* **39**, 1491-1494, 2004.

[60] RP Singh, S Kumar, and CSP Ojha. Nutrient requirement for UASB process: a review. *Biochemical Engineering Journal* **3**, 35–54, 1999.

[61] N Kosaric, R Blaszczyk, and L Orphan. Factors influencing formation and maintenance of granules in upflow anaerobic sludge blanket reactors (UASBR). *Water Science and Technology* **22**, 275–282, 1990.

[62] DJ Batstone, J Keller, I Angelidaki, SV Kalyuzhnyi, SG Pavlostathis, A Rozzi, WTM Sanders, H Siegrist, and V Vavilin. *Anaerobic Digestion Model No 1 (ADM1).* IWA Publishing, London, UK, 2002.

[63] RC Jin, C Ma, Q Mahmood, GF Yang, and P Zheng. Anammox in a UASB reactor treating saline wastewater. *Process Safety and Environmental Protection* **89**, 342–348, 2011.

[64] Y Chen, JJ Cheng, and KS Creamer. Inhibition of anaerobic digestion process: A review. *Bioresource Technology* **99**, 4044-4064, 2008.

[65] SJ Lim and TH Kim. Applicability and trends of anaerobic granular sludge treatment process. *Biomass and Bioenergy* **60**, 189-202, 2014.

[66] WJ Parker. Application of the ADM1 model to advanced anaerobic digestion. *Bioresource Technology* **96**, 1832-1842, 2005.

[67] IWA task group for mathematical modelling of anaerobic digestion processes. *Anaerobic digestion model no.1 (ADM1)*, Scientific and Technical report No. 13, IWA Publishing, London, 2002.

[68] L Yu, Q Zhao, J Ma, C Frear, and S Chen. Experimental and modeling study of a two-stage pilot scale high solid anaerobic digester system. *Bioresource Technology* **124**, 8-17, 2012.

[69] DJ Batstone, JLA Hernandez, and JE Schmidt. Hydraulics of laboratory and full-scale upflow anaerobic sludge blanket (UASB) reactors. *Biotechnology and Bioengineering* **91**, 387–391, 2005.

[70] TT Ren, Y Mu, BJ Ni, and HQ Yu. Hydrodynamics of upflow anaerobic sludge blanket reactors. *AIChE Journal* **55**, 516–528, 2009.

[71] R Rodriguez Gomez, G Renmao, L Moreno, and L Liu. A model to describe the performance of the UASB reactor. *Biodegradation* **25**, 239-251, 2014.

[72] Y Chen, J He, Y Mu, YC Huo, Z Zhang, TA Kotsopoulos, and RJ Zeng. Mathematical modelling of upflow anaerobic sludge blanket (UASB) reactors: Simultaneous accounting for hydrodynamics and bio-dynamics. *Chemical Engineering Science* **137**, 677-684, 2015.

[73] O Bernad, Z Hadj-Sadok, D Dochain, A Genovesi, and JP Steyer. Dynamical model development and parameter identification for an anaerobic wastewater treatment process. *Biotechnology and Bioengineering* **75**(4), 424–438,2001.

[74] I Lopez and L Borzacconi. Modelling a full scale UASB reactor using a COD global balance approach and state observers. *Chemical Engineering Journal* **146**, 1-5, 2009.

[75] S Kalyuzhnyi, V Fedorovich, and P Lens. Dispersed plug flow model for upflow anaerobic sludge bed reactors with focus on granular sludge dynamics. *Journal of Industrial Microbiology and Biotechnology* **33**, 221-237, 2006.

[76] D Sponza and M Isik. Substrate removal kinetics in an upflow anaerobic sludge blanket reactor decolorizing simulated textile wastewater. *Process Biochemistry* **40**, 1189-1198, 2005.

[77] B Demirel, O Yenigun, and TT Onay. Anaerobic treatment of dairy wastewaters: a review. *Process Biochemistry* **40**(8), 2583-2595, 2005.

[78] JLR Gutiérrez, PAG Encina, and FF Polanco. Anaerobic treatment of cheese-production wastewater using UASB reactor. *Bioresource Technology* **37(3)**, 271-276, 1991.

[79] F Carvalho, AR Prazeres, and J Rivas. Cheese whey wastewater: Characterization and treatment. *Science of the Total Environment* **445-446**, 385-396, 2013.

[80] W Janczukowicz, M Zielinski, and M Dbowski. Biodegradability evaluation of dairy effluents originated in selected sections of dairy production. *Bioresource Technology* **99(10)**, 4199-4205, 2008.

[81] AR Prazeres, F Carvalho, and J Rivas. Cheese whey management: A review. *Journal of Environmental Management* **110**, 48-68, 2012.

[82] Angie's Option, https://www.angiesoption.com/2017/05/whey-protein-supplements/whey, 2017.

[83] Michaelis. *Illustrated dictionary- Portuguese-English G-Z.* IV, Ediçoes Melhoramentos, Lisboa, Portugal, 1995.

[84] E Castelló, CG Santos, T Iglesias, G Paulino, J Wenzel, L Borzacconi, and C Etchebehere. Feasibility of biohydrogen production from cheese whey using a UASB reactor: links between microbial community and reactor performance. *International Journal of Hydrogen Energy* **34(14)**, 5674-5682, 2009.

[85] CD Pereira, O Diaz, and A Cobos. Valorization of by-products from ovine cheese manufacture: clarification by thermocalcic precipitation/microfiltration before ultrafiltration. *International Dairy Journal* **12(9)**, 773-783, 2002.

[86] H Escalante, L Castro, MP Amaya, L Jaimes, and J Jaimes-Estévez. Anaerobic digestion of cheese whey: Energetic and nutritional potential for the dairy sector in developing countries. *Waste Management* **71**, 711-718, 2018.

[87] SW Kataki, H West, M Clarke, and DC Baruah. Phosphorous recovery as struvite from farm, municipal and industrial water: Feedstock suitability, methods and pre-treatments. *Waste Management* **49**, 437-454, 2016.

[88] M Kamali and Z Khodaparast. Review on recent developments on pulp and paper mill wastewater treatment. *Ecotoxicology and Environmental Safety* **114**, 326-342, 2015.

[89] M Kamali, T Gameiro, MEV Costa, and I Capela. Anaerobic digestion of pulp and paper mill wastes- An overview of the developments and improvement opportunities. *Chemical Engineering Journal* **298**, 162-182, 2016.

[90] Valmet, https://www.valmet.com/media/articles/all-articles/suzano-sets-new-standards-for-green-energy-in-brazil, 2018.

[91] H Holik (Ed.). *Handbook of Paper and Board.* Wiley-VCH, Weinheim, 2006.

[92] J Hong and X Li. Environmental assessment of recycled printing and writing paper: a case study in China. *Waste Management* **32(2)**, 264-270, 2012.

[93] PS Wiegand, CA Flinders, GG Ice, DJH Sleep, BJ Malmberg, and I Lama. Water profiles of the forest products industry and their utility in sustainability assessment. *Tappi Journal* 19-27, July 2011.

[94] DS Fraser, K O'Halloran, and MR Van den Heuvel. Toxicity of pulp and paper solid organic waste constituents to soil organisms. *Chemosphere* **74**, 660-668, 2011.

[95] ND Park, SS Helle, and RW Thring. Combined alkaline and ultrasound pretreatment of thickened pulp mill waste activated sludge for improved anaerobic digestion. *Biomass Bioenergy* **46**, 750–756, 2012.

[96] JD Browne, E Allen, and JD Murphy. Improving hydrolysis of food waste in a leach bed reactor. *Waste Management* **33**, 2470-2477, 2013.

[97] Zero Waste Europe, Reporting the International Training on Organics Management, https://zerowasteeurope.eu/2014/10/international-training-on-organics-management, 2014.

[98] L De Baere, B Mattheeuws, and F Velghe. *State of the art of anaerobic digestion in Europe.* In: IWA 12th World Congress on Anaerobic Digestion, October 31-November 4, Guadalajara, Mexico, 2010.

[99] A Davidsson, C Gruvberger, TH Christensen, TL Hansen, and JIC Jansen. Methane yield in source-sorted organic fraction of municipal solid waste. *Waste Management* **27**, 406-414, 2007.

[100] CJ Banks, M Chesshire, S Heaven, and R Arnold. Anaerobic digestion of source-segregated domestic food waste: performance assessment by mass and energy balance. *Bioresource Technology* **102**, 612-620, 2011.

[101] R Campuzano and S Gonzalez-Martinez. *Biogas production from enzymatically pretreated Mexico City's organic waste*, 4th International Symposium on Energy from Biomass and Waste, 12-15 November 2012, Cini Foundation, Venice, Italy.

[102] JD Browne and JD Murphy. The impact of increasing organic loading in two phase digestion of food waste. *Renewable Energy* **71**, 69-76, 2014.

[103] JY Wang, H Zhang, O Stabnikova, SS Ang, and JH Tay. A hybrid anaerobic solid-liquid system for food waste digestion. *Water Science & Technology* **52**, 223-228, 2005.

[104] GN Demirer and S Chen. Anaerobic biogasification of undiluted dairy manure in leaching bed reactors. *Waste Management* **28**, 112-119, 2008.

[105] SE Nayono, C Gallert, and J Winter. Co-digestion of press water and food waste in a biowaste digester for improvement of biogas production. *Bioresource Technology* **101**, 6987-6993, 2010.

[106] FAO Source. 2000-2002 world beer production. BIOS Int. **8**(2):47-50, 2003.

[107] T Wainwright. Basic Brewing Science. Magicprint (Pty) Ltd., South Africa, 1998.

[108] https://phys.org/news/2016-10-brewery-wastewater-energy-storage.html.

[109] AA Olajire. The brewing industry and environmental challenges. *Journal of Cleaner Production*, 1-21, 2012.

[110] Hannover. Best Available Techniques Reference Document on the Food and Milk Industry, BMU. Hannover University, 2002.

[111] https://byo.com/mr-wizard/what-does-whirlpooling-your-beer-mean-and-how-do-you-do-it/, 2018.

[112] MI Baloch, JC Akunna, PJ Collier. The performance of a phase separated granular bed bioreactor treating brewery wastewater. Bioresource Technology 98, 1849-1855, 2007.

[113] GD Zupancic, M Ros, M Klemencic, M Oset, R Marinsek-Logar. Biogas production from brewery yeast in an EGSB reactor. *Brauwelt Intl.* **34**, 108-113, 2016.

[114] SI Mussatto. Brewer's spent grain: a valuable feedstock for industrial applications. *Journal of the Science of Food and Agriculture* **94**, 1264–1275, 2013.

[115] L Fillaudeau, P Blanpain-Avet, G Daufin. Water, wastewater and waste management in brewing industries. *Journal of Cleaner Production* **14**, 463-471, 2006.

[116] https://www.feedipedia.org/node/74, 2018.

[117] http://frankenmuthbrewery.com/ingredient-yeast/, 2018.

[118] U Behmel, G Leupold, S Vieweger. Production of biogas from vegetable wastes. I. Optimized hydrolysis of the lignocellulosic components in spent grain. *Chem. Mikrobiol. Technol. Leb.* **15**, 55-61, 1993

[119] SI Mussatto, G Dragone, IC Roberto. Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science* **43**, 1-14, 2006.

[120] M Panjicko, GD Zupancic, L Fanedl, RM Logar, M Tisma, B Zelic. Biogas production from brewery spent grain as a mono-substrate in a two-stage process composed of solid-state anaerobic digestion and granular biomass reactors. *Journal of Cleaner Production* **166**, 519-529, 2017.

[121] I Ruiz, MC Veiga, P de Santiago, and R Blazquez. Treatment of slaughterhouse wastewater in a UASB reactor and anaerobic filter. *Bioresource Technology* **60**, 251-258, 1997.

[122] ABG Valladao, DMG Freire, and MC Cammarota. Enzymatic pre-hydrolisis applied to the anaerobic treatment of effluents from poultry slaughterhouses. *International Biodeterioration & Biodegradation* **60**, 219-225, 2007.

[123] Z Wang and CJ Banks. Evaluation of a two stage anaerobic digester for the treatment of mixed abattoir wastes. *Process Biochemistry* **38**, 1267-1273, 2002.

[124] MI Ahmad, O Ejaz, A Ali, MAQJ Durrani, and IA Khan. Anaerobic digestion of waste from a slaughterhouse. *Journal of Environmental Chemical Engineering* **2**, 1317-1320, 2014.

[125] Abbanoa S.p.A., http://www.abbanoa.it, 2018.

[126] I Mouzakis, FM Pellera, and E Gidarakos. Slaughterhouse by-products treatment using anaerobic digestion. *Waste Management* **71**, 652-662, 2018.

[127] I Angelidaki and BK Ahring. Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Applied Microbiology and Biotechnology* **37**, 808–812, 1992.

[128] A Hejnfelt and I Angelidaki. Anaerobic digestion of slaughterhouse by-products. *Biomass and Bioenergy* **33**, 1046-1054, 2009.

[129] NA Oz and AC Uzun. Ultrasound pretreatment for enhanced biogas production from olive mill wastewater. *Ultrasonics Sonochemistry* **22**, 565-572, 2015.

[130] A Tiehm, K Nickel, M Zellhorn, and U Neis. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. *Water Research* **35**, 2003-2009, 2001.

[131] M Weemaes and W Verstraete. Evaluation of current wet sludge disintegration techniques. *Journal of Chemical Technology and Biotechnology* **73**, 83-92, 1998.

[132] Wikipedia, https://commons.wikimedia.org, 2018.

[133] O Hamdaoui, F Saoudi, M Chiha, and E Naffrechoux. Sorption of malachite green by a novel sorbent, dead leaves of plane tree: equilibrium and kinetic. *Chemical Engineering Journal* **143**, 73-84, 2008.

[134] I Grcic, M Obradovic, D Vujevic, and N Koprivanac. Sono-Fenton oxidation of formic acid/formate ions in an acqueous solution: From an experimental design to the mechanistic modelling. *Chemical Engineering Journal* **164**, 196-207, 2010.

[135] YG Adewuyi. Sonochemistry: environmental science and engineering applications. *Industrial & Engineering Chemistry Research* **40**, 4681-4715, 2001.

[136] CN Gajendragadkar and PR Gogate. Intensified recovery of valuable products from whey by use of ultrasound in processing steps- A review. *Ultrasonics Sonochemistry* **32**, 102-118, 2016.

[137] Tolmezzo News, https://tolmezzo.wordpress.com, 2010.

[138] R Campuzano and S Gonzalez-Martinez. Extraction of soluble substances from organic solid municipal waste to increase methane production. *Bioresource Technology* **178**, 247-253, 2015.

[139] MA Voelklein, A Jacob, R O'Shea, and JD Murphy. Assessment of increasing loading rate on two-stage digestion of food waste. *Bioresource Technology* **202**, 172-180, 2016.

[140] M Mainardis, V Cabbai, G Zannier, D Visintini, and D Goi. Characterization and BMP tests of liquid substrates for high-rate anaerobic digestion. *Chemical and Biochemical Engineering Quarterly* **31(4)**, 451-460, 2017.

[141] APHA. *Standard methods for the Examination of Water and Wastewater.* 22nd ed. American Public Health Association, America Water Works Association, Water Environment Federation, Washington DC, 2012.

[142] M Dubois, KA Gilles, JK Hamilton, PA Rebers, and F Smith. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350-356, 1956.

[143] DA Rouch, H Roginski, ML Britz, and P Roupas. Determination of a nitrogen conversion factor for protein content in Cheddar cheese. *International Dairy Journal* **18**, 216-220, 2008.

[144] V Cabbai, M Ballico, E Aneggi, and D Goi. BMP tests of source selected OFMSW to evaluate anaerobic codigestion with sewage sludge. *Waste Management* **33**, 1626-1632, 2013.

[145] X Li, S Guo, Y Peng, Y He, S Wang, L Li, and M Zhao. Anaerobic digestion using ultrasound as pretreatment approach: Changes in waste activated sludge, anaerobic digestion performances and digestive microbial populations. *Biochemical Engineering Journal* **139**, 139-145, 2018.

[146] Dr Hielscher, https://www.hielscher.com, 2018.

[147] MNI Siddique and ZA Wahid. Achievements and perspectives of anaerobic co-digestion: A review. *Journal of Cleaner Production* **194**, 359-371, 2018.

[148] F Kargi and S Uzunçar. Simultaneous hydrogen gas formation and COD removal from cheese whey wastewater by electrohydrolysis. *International Journal of Hydrogen Energy* **37**, 11656-11665, 2012.

[149] N Brown, J Guttler, and A Shilton. Overcoming the challenges of full scale anaerobic co-digestion of casein whey. *Renewable Energy* **96**, 425-432, 2016.

[150] TH Erguder, U Tezel, E Guven, and GN Demirer. Anaerobic biotransformation and methane generation potential of cheese whey in batch and UASB reactors. *Waste Management* **21**, 643-650, 2001.

[151] V Blonskaja and T Vaalu. Investigation of different schemes for anaerobic treatment of food industry wastes in Estonia. *Proceedings of the Estonian Academy of Sciences. Chemistry* **55(1)**, 14-28, 2006.

[152] R Campuzano and S Gonzalez-Martinez. Influence of process parameters on the extraction of soluble substances from OFMSW and methane production. *Waste Management* **62**, 61-68, 2017.

[153] SE Nayono, J Winter, and C Gallert. Anaerobic digestion of pressed off leachate from the organic fraction of municipal solid waste. *Waste Management* **30**, 1828-1833, 2010.

[154] C Bougrier, D Dognin, C Laroche, V Gonzalez, D Benali-Raclot, and JAC Rivero. Anaerobic digestion of Brewery Spent Grains: Trace elements addition requirement. *Bioresource Technology* **247**, 1193-1196, 2018.

[155] WF Owen, DC Stuckey, JB Healy, LY Young, and PL McCarty. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research* **13**, 485-492, 1979.

[156] A Ware and N Power. Modelling methane production kinetics of complex poultry slaughterhouse wastes using sigmoidal growth functions. *Renewable Energy* **104**, 50-59, 2017.

[157] B Wang, IA Nges, M Nistor, and J Liu. Determination of methane yield of cellulose using different experimental setups. *Water Science & Technology* **70(4)**, 599-604, 2014.

[158] J De Vrieze, L Raport, B Willems, S Verbrugge, E Volcke, E Meers, LT Angenent, and N Boon. Inoculum selection influences the biochemical methane potential of agro-industrial substrates. *Microbial Biotechnology* **8(5)**, 776-786, 2015.

[159] I Angelidaki, M Alves, D Bolzonella, L Borzacconi, JL Campos, and AJ Guwy. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science and Technology* **59**, 927-934, 2009.

[160] F Raposo, MA De la Rubia, V Fernandez-Cegri, and R Borja. Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. *Renewable & Sustainable Energy Reviews* **16**, 861-877, 2012.

[161] HL Gough, D Nelsen, C Muller, and J Ferguson. Enhanced methane generation during thermophilic co-digestion of confectionary waste and grease-trap fats and oils with municipal wastewater sludge. *Water Environment Research* **85**, 175-183, 2013.

[162] M Lesteur, V Bellon-Maurel, C Gonzalez, E Latrille, JM Roger, G Junqua, and JP Steyer. Alternative methods for determining anaerobic biodegradability: A review. *Process biochemistry* **45**, 431-440, 2010.

[163] K Koch, YB Fernandez, and JE Drewes. Influence of headspace flushing on methane production in Biochemical Methane Potential (BMP) tests. *Bioresource Technology* **186**, 173-178, 2015.

[164] Veneto uno, http://www.venetouno.it/notizia/31378/incendio-alla-cartiera-giorgione-di-castelfranco, 2012.

[165] MO Fagbohungbe, BMJ Herbert, L Hurst, CN Ibeto, H Li, SQ Usmani, and KT Semple. The challenges of anaerobic digestion and the role of biochar in optimizing anaerobic digestion. *Waste Management* **61**, 236-249, 2017.

[166] NM Sunyoto, M Zhu, Z Zhang, and D Zhang. Effect of biochar addition on hydrogen and methane production in two-phase anaerobic digestion of aqueous carbohydrates food waste. *Bioresource Technology* **219**, 29-36, 2016.

[167] https://www.ethz.ch/content/main/de/news-und-veranstaltungen/eth-news/news/2014/04/biochar-is-there-a-dark-side.html, 2018.

[168] JH Ko, N Wang, T Yuan, F Lu, P He, and Q Xu. Effect of nickel-containing activated carbon on food waste anaerobic digestion. *Bioresource Technology* **266**, 516-523, 2018.

[169] SA Opatokun, V Strezov, and T Kan. Product based evaluation of pyrolysis of food waste and its digestate. *Energy* **92(3)**, 349-354, 2015.

[170] FC Luz, S Cordiner, A Manni, V Mulone, V Rocco, R Braglia, and A Canini. Ampelodesmos mauritanicus pyrolysis biochar in anaerobic digestion process: Evaluation of the biogas yield. *Energy* **161**, 663-669, 2018.

[171] D Bolzonella, F Fatone, P Pavan, and F Cecchi. Anaerobic fermentation of organic municipal solid wastes for the production of soluble organic compounds. *Industrial & Engineering Chemistry Research* **44(10)**, 3412-3418, 2005.

[172] RA Labatut, LT Angenent, and NR Scott. Biochemical methane potential and biodegradability of complex organic substrates. *Bioresource Technology* **102**, 2255-2264, 2011.

[173] M Zielinski, M Debowski, A Grala, and M Dudek. Wplyw wstepnej ultradzwiekowej sonifikacji serwatki kwasnej na efektywnosc procesu fermentacji metanowej. *Srodkowo-Pomorskie Towarzystwo Naukowe Ochrony Srodowiska* **14**, 471-480, 2012.

[174] G Nazimudheen, K Roy, T Sivasankar, and VS Moholkar. Mechanistic investigations in ultrasonic pretreatment and anaerobic digestion of landfill leachates. *Journal of Environmental Chemical Engineering* **6(2)**, 1690-1701, 2018.

[175] F Passos, S Astals, and I Ferrer. Anaerobic digestion of microalgal biomass after ultrasound pretreatment. *Waste Management* **34(11)**, 2098-2103, 2014.

[176] BR Dhar, G Nakhla, and MB Ray. Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge. *Waste Management* **32(3)**, 542-549, 2012.

[177] T Meyer and EA Edwards. Anaerobic digestion of pulp and paper mill wastewater and sludge. *Water Research* **65**, 321-349, 2014.

[178] M Panjicko, GD Zupancic, B Zelic. Anaerobic biodegradation of raw and pre-treated brewery spent grain utilizing solid state anaerobic digestion. *Acta Chimica Slovenica* **62**, 818-827, 2015.

[179] V Cabbai. *Energetic conversion of Organic Fraction of Municipal Solid Waste by Anaerobic co-digestion with sewage sludge.* Ph.D. Thesis in Chemical and Energy Technologies, 2014.

[180] GK Kafle and L Chen. Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. *Waste Management* **48**, 492-502, 2016.

[181] GD Gioannis, A Muntoni, G Cappai, and S Milia. Landfill gas generation after mechanical biological treatment of municipal solid waste: estimation of gas generation rate constants. *Waste Management* **29(3)**, 1026-1034, 2009.

[182] GK Kafle and SH Kim. Anaerobic treatment of apple waste with swine manure for biogas production: batch and continuous operation. *Applied Energy* **103**, 61-72, 2013.

[183] P Parameswaran and BE Rittmann. Feasibility of anaerobic co-digestion of pig waste and paper sludge. *Bioresource Technology* **124**, 163-168, 2012.

[184] AM Gibson, N Bratchell, and TA Roberts. The effect of sodium chloride and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry. *Journal of Applied Bacteriology* **62(6)**, 479–490, 1987.

[185] C Rico, N Munoz, J Fernàndez, and JL Rico. High-load anaerobic co-digestion of cheese whey and liquid fraction of dairy manure in a one-stage UASB process: Limits in co-substrates ratio and organic loading rate. *Chemical Engineering Journal* **262**, 794-802, 2015.

[186] S Chinnaraj and GV Rao. Implementation of an UASB anaerobic digester at bagasse-based pulp and paper industry. *Biomass and Bioenergy* **30(3)**, 273-277, 2006.

[187] D Goi, G Di Giorgio, I Cimarosti, and G Dolcetti. Prove sperimentali per la determinazione delle costanti cinetiche e stechiometriche in impianti a fanghi attivi: un caso di studio. *Ingegneria Ambientale* **XXXIII**, 348-355, 2004.

[188] H Escalante, L Castro, V Besson, and J Jaimes-Estévez. Feasibility of the anaerobic process of Cheese Whey in a plug flow reactor (PFR). *Ingenierìa, Investigaciòn y Tecnologìa* **VWIII**, 265-277, 2017.

[189] L Castro, H Escalante, J Jaimes-Estevez, LJ Diaz, K Vecino, G Rojas, L Mantilla. Low cost digester monitoring under realistic conditions: Rural use of biogas and digestate quality. *Bioresource Technology* **239**, 311-317, 2017.

[190] YH Song, GL Qiu, P Yuan, XY Cui, JF Peng, P Zeng, and F Qian. Nutrients removal and recovery from anaerobically digested swine wastewater by struvite crystallization without chemical additions. *Journal of Hazardous Materials* **190**, 140-149, 2011.

[191] R Nkoa. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agronomy Sustainable Development* **34(2)**, 473-492, 2014.

[192] L Appels, J Baeyens, J Degrève, and R Dewil. Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science* **34**, 755-781, 2008.

[193] P Stolzenburg, A Capdevielle, S Teychené, and B Béatrice. Struvite precipitation with MgO as a precursor: Application to wastewater treatment. *Chemical Engineering Science* **133**, 9-15, 2015.

[194] S Uludag-Demirer, GN Demirer, and S Chen. Ammonia removal from anaerobically digested dairy manure by struvite precipitation. *Process Biochemistry* **40**, 3667-3674, 2005.

[195] T Karak, I Sonar, JR Nath, RK Paul, S Das, RK Boruah, and K Das. Struvite for composting of agricultural wastes with termite mound: Utilizing the unutilized. *Bioresource Technology* **187**, 49-59, 2015.

[196] N Powell, A Broughton, C Pratt, and A Shilton. Effect of whey storage on biogas produced by co-digestion of sewage sludge and whey. *Environmental Technology* **34(19)**, 2743-2748, 2013.

[197] K Yetilmezsoy, F Ilhan, E Kocak, and H Akbin. Feasibility of struvite recovery process for fertilizer industry: A study of financial and economic analysis. *Journal of Cleaner Production* **152**, 88-102, 2017.

[198] K Heppner. Granular Phosphorus Extracted from Hog Manure Shows Promise in Canola & Wheat, https://www.realagriculture.com, 2016.

[199] Whey cheese machinery & equipment, http://www.gidamaksan.com, 2018.

[200] M Mainardis, S Flaibani, M Trigatti, D Goi. Techno-economic feasibility of Cheese Whey Anaerobic Digestion in small Italian dairies and effect of Ultra-Sound pre-treatment on BMP yield. *in progress.*

[201] http://www.snam.it, 2016.

[202] Cogengreen S.A., http://www.cogengreen.com/en/75-402-kw-natural-gas-fired-ecogen-chp-units-natural-gas-fired-condensing-micro-and-mini-chp-units, 2018.

[203] N Palmieri, MB Forleo, E Salimei. Environmental impact of a dairy cheese chain including whey feeding: An Italian case study. *Journal of Cleaner Production* **140**, 881-889, 2017.

[204] L Paulo de Lima, GBD Ribeiro, R Perez. The energy mix and energy efficiency analysis for Brazilian dairy industry. *Journal of Cleaner Production* **181**, 209-216, 2018.

[205] Autorità di Regolazione per Energia, Reti e Ambiente, https://www.arera.it, 2018.

[206] S Ganju and PR Gogate. A review on approaches for efficient recovery of whey proteins from dairy industry effluents. *Journal of Food Engineering* **215**, 84-96, 2017.

[207] R Bozanic, I Barukcic, K Lisak, Jakopovic, and L Tratnik. Possibilities of whey utilisation. *Austin Journal of Nutrition and Food sciences* **2**, 1036-1042, 2014.

[208] MA Levin, KJ Burrington, and RW Hartel. Composition and functionality of whey protein phospholipid concentrate and delactosed permeate. *Journal of Dairy Science* **99**, 1-11, 2016.

[209] Supplements SA Whey Protein Concentrate, https://www.supplementworld.co.za, 2018.

[210] D Barba, F Beolchini, D Cifoni, F Vegilo. Whey protein concentrate production in a pilot scale two-stage diafiltration process. *Separation Science and Technology* **36(4)**, 587-603, 2001.

[211] A Gharsallaoui, G Roudaut, O Chambin, A Voilley, and R Saurel. Applications of spray-drying in microencapsulation of food ingredients: an overview. *Food Research International* **40(9)**, 1107-1121, 2007.

[212] Axium process, https://axiumprocess.com.

[213] A Sosnik and KP Seremeta. Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers. *Advances in Colloid and Interface Science* **223**, 40-54, 2015.

[214] H Sabarez. *Drying of Food Materials.* CSIRO Food and Nutrition. Elsevier, Werribee Australia, 2016.

[215] C Anandharamakrishnan, CD Rielly, and AGF Stapley. Spray-freeze-drying of whey proteins at sub-atmospheric pressures. *Dairy Science and Technology* **90**, 321-334, 2010.

[216] IEA. Task 37 biogas country overview. International Energy Agency Bioenergy Task 37. http://www.iea-biogas.net/files/daten-redaktion/download/publications/country-reports/november 2013/Countryreport2013.pdf, 2014.

[217] F. Cecchi, P. Pavan, D. Bolzonella, S. Mace, and J. Mata-Alvarez. Anaerobic digestion of the organic fraction of municipal solid waste for methane production: research and industrial application. *Comprehensive Biotechnology*, 2011.

[218] J Mata-Alvarez, J Dosta, S Macé, and S Astals. Codigestion of solid wastes: a review of its uses and perspectives including modelling. *Critical Reviews in Biotechnology* **31(2)**, 99-111, 2011.

[219] A Kubule, L Zogla, J Ikaunieks, and M Rosa. Highlights on energy efficiency improvements: a case of a small brewery. *Journal of Cleaner Production* **138**, 275-286, 2016.

[220] B Muster-Slawitsch, H Hubmann, M Murkovic, and C Brunner. Process modelling and technology evaluation in brewing. *Chemical Engineering and Processing: Process Intensification* **84**, 98-108, 2014.

Conferences and publications

This Ph.D. was particularly interesting because of the opportunity to share knowledge with highly specialized colleagues, both on National and European level; moreover, it gave me the opportunity to get in touch with high-level professionals, from which I learned a lot.

Publications

- M. Mainardis, S. Flaibani, M. Trigatti, D. Goi, "**Techno-economic feasibility of Cheese Whey Anaerobic Digestion in small Italian dairies and effect of Ultra-Sound pre-treatment on BMP yield**", *in progress.*

- M. Mainardis, V. Cabbai, G. Zannier, D. Goi, "**Characterization and BMP tests of liquid substrates for high-rate anaerobic digestion**". Chemical and Biochemical Engineering Quarterly 31(4), 2017.

- M. Mainardis, G. Zannier, M. Mion, D. Visintini, D. Goi,"**Energetic Valorisation of Cheese Whey using UASB Technology: a case study**", Communications in agricultural and applied biological sciences-Special Issue : Advances & Trends in Biogas and Biorefineries-Vol: 82 (4) 1 - 190 (2017).

- M. Mainardis, V. Cabbai, D. Goi, "**UASB anaerobic treatment and OFMSW reutilization: Tolmezzo case potentiality**", Friulian Journal of Science 20, 2016.

Oral presentations at conferences

- M. Mainardis, N. De Bortoli, M. Mion, V. Cabbai, D. Goi. "**Thermo-economic evaluation of combined heat and power generation in wastewater treatment plant to optimize sludge drying**". Proceedings in Sludge Management In Circular Economy (SMICE) Conference, 23-25 May 2018, Rome.
- M. Mainardis, M. Mion, G. Zannier, D. Goi. "**A territory-oriented approach to improve high-loaded liquid waste management: the case-study of Tolmezzo (Ud)**". Proceedings in Young Water Professionals 2018 Conference, 07-11 May 2018, Zagreb, Croatia.

Poster sessions

- M. Mainardis, M. Mion, G. Zannier, D. Goi, "**UASB anaerobic digestion of highly-loaded liquid substrates: a pilot-study in Friuli-Venezia Giulia region**", Proceedings in EBA (European Biogas Association) 2018 conference, 24-26 January 2018, Antwerp (Belgium).
- M. Mainardis, G. Zannier, M. Mion, D. Goi, "**UASB anaerobic treatment of liquid substrates: A case study in Friuli-Venezia Giulia region**", Proceedings of 9th Eastern European Young Water Professionals Conference, Book of Abstracts, ISBN 978-963-313-256-2, 24-27 May 2017, Budapest (Hungary).
- M. Mainardis, G. Zannier, D. Goi, "**Selecting liquid substrates for UASB process upgrade**": Characterization and BMP tests", Proceedings in EBA (European Biogas Association) 2016 conference, 26-30 September 2016, Ghent (Belgium).
- M. Mainardis, G. Rossi, V. Cabbai, D. Goi, "**Characterization of high-loaded organic substrates and suitability as a potential feed for high-velocity UASB reactors**", Proceedings of SIDISA (Simposio Italo-Brasiliano di Ingegneria Sanitaria Ambientale) 2016, Book of Abstracts, ISBN 978-88-496-391-1, 20-23 June 2016, Rome.

Teaching activities

- **Co-relator** for the following Master's degree "Engineering for Energy and

Environment" dissertations:

1. Energetic recovery from Cheese Whey using Anaerobic Digestion: A case study in Friuli-Venezia Giulia plain, **Dott. Simone Flaibani**, A.Y. 2017/2018;
2. Energy recovery from brewery through AD in the mountain area of Friuli-Venezia Giulia region, **Dott. Fabio Mazzolini**, A.Y. 2017/2018.

- Oral presentation, "**Perspectives and innovation in IWS in Fvg: CAFC S.p.A. and Poiana S.p.A. case studies- Energy and material recovery**", World Water Day 2018, Udine, 22 March 2018.
- Seminar lecture: "**Sustainability & innovation in the IWS: a *territory-oriented* approach**", Secondary school ISIS Solari (Tolmezzo, Ud), 06 February 2018.
- Oral presentation, "**UASB anaerobic treatment of liquid substrates: Tolmezzo case study**", 16th Annual Congress of Scientific and Technological Friulian Society, Capriva (Gorizia), 18 November 2017.
- Seminar lecture: "**Integrated Water Service management in Friuli-Venezia Giulia region**", Ohio Study University study abroad, Udine, July 2017.
- Seminar lecture: "**Integrated Water Service: from water withdrawal for potable uses to wastewater treatment**", Udine event "Conoscenza in festa", June 2017.
- Seminar lecture: "**Anaerobic digestion: Principles and applications**", Master Degree in "Engineering for Environment and Territory" and "Engineering for environment and energy", June 2016

Workshops, Summer schools and Seminars

- ATS, "**SMART- Plant Horizon2020 European Innovation Action**", Carbonera (Tv), 08 February 2018.
- IRES, "**Integrated water cycle lessons**", University of Udine, November 2017-January 2018.

- CAFC S.p.A., “**IWS- Water Safety Plan: Cooperation between authorities**”, Udine, 7 December 2017.
- CAFC S.p.A., “**WW discharge in light of Water Protection Plan of Fvg Region and of CAFC sewage system regulation**”, Udine, 24 November 2017.
- SEAM Engineering, “**Control systems in WWTPs**”, Lomazzo (Como), 27 October 2017.
- CAFC S.p.A., “**IWS-WWT sludge: reality roadmap**”, Lonigo (Vicenza), 23 September 2017.
- University of Pavia, Summer school “**Energetic and material recovery issues in modern urban metabolism: strategies and technologies for a sustainable future**”, Lake Como school of Advanced Studies, 21-25 August 2017.
- IRES, “**Integrated water cycle lessons**”, University of Udine, February-March 2017.
- DHI Group in Genova, Practical workshop “**Advanced Modelling of WWTP with WEST**”, November 2016.
- **EcoMondo** fair in Rimini, November 2016.
- University of Udine, Round table “**Biomethane**”, 6 October 2016.
- University of Pavia, Summer school “**Sustainable Water- Energy- Centric Communities (SWEC)**”, Lake Como school of Advanced Studies, 9-13 May 2016.
- Polytechnic of Milan, “**Technical innovations for energy and resources recovery from agro-industrial wastes**”, Milan, 5 October 2015.

This Ph.D. was encouraged by the agreement between University of Udine and the society Carniacque S.p.A. (Integrated Water Service authority for the mountain area of Friuli-Venezia Giulia region), through the activation of a "High education and research apprenticeship" contract. Carniacque S.p.A. was then integrated into CAFC S.p.A.; the research project was funded by POR FESR 2007-2013 of Friuli-Venezia Giulia region (LR 47/78) development program. All the people involved in this Ph.D. program recognized that such collaborations should be encouraged, because they can represent a way to develop research topics and increase specific know-how, shared between academia and companies.

Acknowledgements

I would like to thank all the people that supported my studies and activities during these 3 years. I will start from my family, that was always ready to help me in the most difficult periods.

Secondly, I would acknowledge my supervisor, Prof. Daniele Goi, for his good guidance and availability for discussions and reviews. Also, I want to thank all the research group in "Environmental Pollution and Treatment", namely dott. Eleonora Aneggi, eng. Matteo Trigatti, eng. Roberto Perin, eng. Valentina Cabbai, dott. Giancarlo Mio, dott. Andrea Fattori, dott. Simone Flaibani, dott. Fabio Mazzolini, eng. Alì Khakbaz, eng. Sajid Hussain.

Thirdly, I want to thank Carniacque S.p.A. (and then CAFC S.p.A.) colleague, for all the time that we passed together, the help that I received in the different activities of the research project and the specialized knowledge that I could gain through this interesting experience. I will name just some of them: dott. Fabrizio Luches, eng. Giampaolo Zannier, p.i. Deborah Deotto, p.i. Micol Vera Ballarini, Marco Del Negro, Luca Cassutti, Luca Scarabelli, Manuel Piazza, ing. Nicola De Bortoli, eng. Riccardo Vazzoler, eng. Gabriele Gallina, eng. Domenico Espro, eng. Francesca Fachin, dott. Miriam Scarsini, Giorgio Iob, Laura Recanati, dott. Christian Rumiz. A special thanks to my CAFC tutor, eng. Michele Mion, and CAFC General Director, eng. Massimo Battiston.

Finally, I acknowledge all my friends, that encouraged my studies and supported each step of my work and everyday life.